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Evaluation of Agar Plates for Direct Enumeration of *Campylobacter* spp. from Poultry Carcass Rinses

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Campy-Cefex, a modification of Campy-Cefex, modified charcoal cefoperazone deoxycholate (mCCDA), Karmali, CAMPY, and Campy-Line agars were evaluated for their efficiency to isolate and enumerate *Campylobacter* spp. from poultry carcass rinses. Campy-Cefex and its modification produced the best results but were statistically similar to CAMPY, mCCDA, and Karmali.

Campylobacter spp. are found at a high prevalence in raw poultry carcasses. Consequently, the mishandling of raw poultry and the consumption of undercooked poultry are important risk factors for human campylobacteriosis (8, 20, 27). Studies in the United States clearly show that more than 80% of commercial chicken carcasses may be positive for *Campylobacter* spp. (16, 23), although negative flocks can also be found. When *Campylobacter* spp. are present, their numbers per milliliter of carcass rinse can vary from 1 to 3 log CFU. This range makes direct enumeration on agar plates an alternative for the rapid identification of *Campylobacter* spp. in carcass rinses.

Several agar plates have been developed or adapted for the identification of *Campylobacter* spp. from enriched poultry samples. However, few have been used for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. Our research goals were to compare the efficacy of five plating media for direct enumeration of *Campylobacter* spp. from poultry carcass rinses and to determine if a modification of Campy-Cefex (mCampy-Cefex), a low-cost medium, was comparable to the original medium.

Twenty postchill carcass rinses were collected per visit from four processing plants. Each plant was visited three times (total, 240 samples; 20 samples/visit from three visits to four plants). Samples were collected using the carcass rinse method (3) and processed within 4 h of collection. Each carcass rinse was plated onto Campy-Cefex agar (26), mCampy-Cefex, modified charcoal cefoperazone deoxycholate agar (mCCDA) (6, 13), Campy-Line agar (17), Karmali agar (15), and CAMPY agar (Table 1). CAMPY agar is also referred to as CampyFDA (14) and is based on the medium developed by Skirrow (25) with the addition of amphotericin B (similar to Campy-BAP) and sodium pyruvate, sodium metabisulfite, and ferrous sulfate (Martin Blaser, New York University School of Medicine, personal communication). Modified Campy-Cefex agar was made by replacing cycloheximide with amphotericin B and laked horse blood with regular whole, lysed horse blood.

For each medium, two plates were each spread with 0.1 ml of the carcass rinse and four plates were each spread with 0.25 ml of the rinse (18, 19). Plates were incubated at 42°C for 48 h in Glad Fresh Protect bags (The Glad Products Company, Oakland, CA) flushed with a microaerophilic gas mixture (BOC Gases, Hixson, TN) containing 10% CO_2, 5% O_2, and 85% N_2. Plates without *Campylobacter* growth after 48 h were incubated an additional 24 h to ensure maximum recovery of *Campylobacter* spp. from samples containing low numbers of cells. Presumptive identification was based on colony morphology, phase contrast microscopy (morphology and motility), and positive results from catalase and oxidase tests (24). Confirmation of isolates was done with a multiplex PCR assay that identifies *Campylobacter jejuni* and *Campylobacter coli* (9). Bacterial DNA was extracted using PrepMan Ultra (Applied Biosystems, Foster City, CA). The PCR assay was followed as described previously (9) with the following changes: the mix was premade (OmniMix HS; Cepheid, Sunnyvale, CA), and the annealing temperature of the amplification cycles was dropped to 47°C. Isolates that gave typical amplicons of *C. coli* with PCR were further confirmed using API Campy tests (bioMérieux. Hazelwood, MO).

Each plant visit was considered a replicate for statistical purposes. Colony counts were converted to $\log_{10}$ CFU/ml and analyzed using the general linear model (GLM) procedure of SAS (SAS Release 8.02; SAS Institute, Inc., Cary, NC). Means were analyzed for differences by Tukey's honestly significant difference test (SAS). Additionally, percent positives were analyzed using GLM procedures. For all tests, a P value of ≤ 0.05 was considered significant.

The combined results from all six plating media showed that 63.3% (152) of all of the samples were positive for *Campylobacter* spp. Campy-Cefex and its modification, mCampy-Cefex, were the media that produced the best results, although there were no statistical differences compared to CAMPY, mCCDA, or Karmali medium. Campy-Line, however, had the lowest counts ($P \leq 0.05$) compared to the other media (Table 2). The results obtained with Campy-Line may be explained by the large numbers of antimicrobial substances incorporated into this medium. Carcass rinses taken postchill have a relatively low level of microbial contamination; therefore, a highly selective medium may not be required.

The modification of Campy-Cefex agar described here performed similarly to Campy-Cefex. This is not surprising, be-

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TABLE 1. Composition of the media used in the experiments

Medium	Base	Supplement
Campy-Cefex	*Brucella* agar[a] (43 g/liter), ferrous sulfate[b] (0.5 g/liter), sodium bisulfite[b] (0.2 g/liter), sodium pyruvate[b] (0.5 g/liter), deionized water (1 liter)	La ked horse blood[c] (50 ml), cefoperazone[b] (33 mg), cycloheximide[b] (0.2 g)
mCampy-Cefex	*Brucella* agar (43 g/liter), ferrous sulfate (0.5 g/liter), sodium bisulfite (0.2 g/liter), sodium pyruvate (0.5 g/liter), deionized water (1 liter)	Lysed horse blood[d] (50 ml), cefoperazone (33 mg), amphotericin B[b] (2 mg)
CAMPY	*Brucella* agar (43 g/liter), deionized water (1 liter)	Lysed horse blood (70 ml), polymyxin B sulfate[e] (1 mg), trimethoprim[b] (0.01 g), vancomycin[b] (0.01 g), amphotericin B (2 mg), novobiocin[b] (0.05 g), sodium pyruvate (0.25 g), sodium metabisulfite (0.25 g), ferrous sulfate (0.25 g)
mCCDA[f]	Nutrient broth no. 2 (25 g/liter), bacteriological charcoal (4 g/liter), casein hydrolysate (3 g/liter), sodium desoxycholate (1 g/liter), ferrous sulfate (0.25 g/liter), sodium pyruvate (0.25 g/liter), agar (12 g/liter), deionized water (1 liter)	Cefoperazone (32 mg), amphotericin B (10 mg)
Karmali[g]	Columbia agar base (39 g/liter), activated charcoal (4 g/liter), hemin (0.032 g/liter), deionized water (1 liter)	Sodium pyruvate (100 mg), cefoperazone (32 mg), vancomycin (20 mg), cycloheximide (100 mg)
Campy-Line	*Brucella* agar (43 g/liter), ferrous sulfate (0.5 g/liter), sodium bisulfite (0.2 g/liter), sodium pyruvate (0.5 g/liter), alpha-ketoglutaric acid[b] (1 g/liter), sodium carbonate[b] (0.6 g/liter), deionized water (1 liter)	Hemin[b] (10 mg), polymyxin B sulfate (0.35 mg), trimethoprim (5 mg), vancomycin (10 mg), cycloheximide (100 mg), cefoperazone (33 mg), triphenyltetrazolium chloride[b] (200 mg)

[a] Acumedia, Lansing, MI.
[b] Sigma-Aldrich, St. Louis, MO.
[c] Oxoid, Inc., New York, NY.
[d] College of Veterinary Medicine, Auburn University.
[e] Alexis Corporation, Lausen, Switzerland.
[f] *Campylobacter* selective blood-free agar (CM0739) and CCDA selective supplement (SR0155, Oxoid).
[g] *Campylobacter* agar base (CM0935) and *Campylobacter* selective supplement (SR0167, Oxoid).

cause the only changes made involved the replacement of the antifungal compound and the type of blood. The addition of blood and/or other ingredients to neutralize the toxic effects of compounds produced in the presence of oxygen and light is common in the formulation of *Campylobacter* media (5). Lysed horse blood was the best supplement out of 22 supplements screened by Bolton and Coates (4) for compounds that facilitate aerotolerance. Amphotericin B has been used to replace cycloheximide in the preparation of *Campylobacter* enrichment broth (Bolton formula) (12). By replacing laked horse blood (Oxoid) with whole lysed horse blood, the price of this medium was reduced considerably (Table 3) without affecting its efficacy for *Campylobacter* sp. recovery. Usually, the supplements are the most expensive components of the media (Table 3).

A combination of two or more media increased the likelihood of detecting *Campylobacter*-positive samples compared to a single medium. However, no statistical differences were seen among combinations. Some of the combinations are exemplified in Table 4. Considering performance, price, and preparation time, the combination of mCampy-Cefex and mCCDA yielded the best results for enumeration of *Campylobacter* spp. from poultry carcass rinses. CAMPY agar consistently exhibited more contaminants that interfered with the recognition of *Campylobacter* colonies. The most prevalent contaminant was *Acinetobacter baumannii*, which grew on all plates except Campy-Line. *Acinetobacter lwoffi* grew in a mixed culture with *A. baumannii* on Karmali. *Pseudomonas* spp. and *Staphylococcus hominis* grew on Campy-Cefex. The identifica-

TABLE 2. *Campylobacter* counts and prevalence by medium

Medium	Mean (log CFU/ml)[a]	Total no. positive (%)
Campy-Cefex	0.66[A]	113 (47.1)[A]
mCampy-Cefex	0.65[A]	118 (49.2)[A]
mCCDA	0.63[A]	109 (45.4)[A]
Karmali	0.53[A]	102 (42.5)[A]
CAMPY	0.51[A]	99 (41.3)[A]
Campy-Line	0.24[B]	51 (21.3)[B]

[a] Pooled standard error of the mean, 0.044. Different letters within a column indicate a significant difference ($P \leq 0.05$).

TABLE 3. Cost of preparation of 1 liter of medium

Plate	Cost of supplies ($)[a]		Labor time (h)	Preparation cost ($)[b]	Total cost per liter ($)
	Base	Supplements			
Campy-Cefex	5.98	20.92	0.40	2.10	28.99
mCCDA	7.25	18.23	0.45	2.36	27.84
Karmali	7.48	13.84	0.45	2.36	23.69
Campy-Line	6.56	7.37	0.61	3.20	17.12
CAMPY	5.61	7.30	0.51	2.65	15.56
mCampy-Cefex	5.98	5.54	0.40	2.10	13.61

[a] Based on prices during spring 2004.
[b] Minimum wage was calculated at $5.25 per hour.

TABLE 4. Results using different plating medium combinations

Plate combination	Average (log CFU/ml)[a]	Total no. positive (%)[a]
Campy-Cefex + CAMPY	0.58[A]	126 (53)[A]
mCampy-Cefex + mCCDA	0.63[A]	133 (55)[A]
Campy-Cefex + mCCDA	0.65[A]	132 (55)[A]
Campy-Cefex + CAMPY + Karmali	0.56[A]	147 (62)[A]
Campy-Cefex + CAMPY + mCCDA	0.59[A]	136 (57)[A]
Campy-Cefex + CAMPY + mCCDA + Karmali	0.58[A]	148 (62)[A]

[a] Pooled standard error of the mean, 0.042. Different letters within a column indicate a significant difference ($P \leq 0.05$).

tion of the contaminants was performed at the National Veterinary Services Laboratories (Ames, IA).

Of the 152 positive samples, the multiplex PCR identified 143 as *C. jejuni*, 3 as *C. coli*, and 6 as containing both *C. jejuni* and *C. coli*. A total of 509 isolates were tested with the multiplex PCR, with 496 (97%) identified as *C. jejuni* and 13 (3%) identified as *C. coli*. *C. coli* isolates grew on mCampy-Cefex (1 isolate), mCCDA (4 isolates), CAMPY (4 isolates) and Karmali (4 isolates). One *C. coli* isolate was found in one visit to plant D, and the rest (12 isolates) were found in a single visit to plant B. Seven samples taken from a single visit to plant B had both *C. jejuni* and *C. coli* growing in different media. Karmali and mCCDA agars that contained a single antibacterial compound (cefoperazone, selective against gram-positives) performed similarly to CAMPY, a medium containing multiple antibacterial compounds (polymyxin and trimethoprim, selective against gram negatives; and vancomycin and novobiocin, selective against gram positives) for isolation of *C. coli*. Consequently, we believe that antimicrobial substances contained in the media are not the only accountable factors involved in the successful isolation of *C. coli*. The discovery that some strains of *C. coli* are more susceptible to cephalothin than *C. jejuni* strains (7, 22) prompted the replacement of cephalothin by cefoperazone in isolation media (10) to increase the probability of isolating *C. coli*. However, further studies are needed to assess the most suitable medium for direct isolation of *C. coli* from poultry carcass rinses.

Direct plating is a valuable technique to study *Campylobacter* spp. in poultry carcasses. Direct plating (18, 19) and a spiral-plating system (23) have been used with success to enumerate *Campylobacter* spp. in postchill carcass rinses. Dickins et al. (11) used concentration and filtration methods with direct plating, without enrichment, to study the genomic diversity of *Campylobacters* isolates from retail poultry carcasses. The results from those studies showed that 67% of the contaminated carcasses had more than one distinguishable pulsed-field gel electrophoresis pattern. In countries where commercial poultry flocks are colonized with a range of bacterial genotypes, such as Australia and the United States (21, 28), direct plating or filtration and direct plating may provide a useful technique to study the genomic diversity of *Campylobacter* spp. These results show that direct plating of carcass rinses is an inexpensive, easy-to-perform enumeration technique for *Campylobacter* spp. A well-validated enumeration technique is useful to assess the impact of intervention strategies aimed at reducing *Campylobacter* spp. in poultry meat, which is an important area of research for regulatory agencies and the poultry industry in the United States and the United Kingdom (1, 2).

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