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Improved protocol for isolation of *Campylobacter* spp. from retail broiler meat and use of pulsed field gel electrophoresis for the typing of isolates

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ABSTRACT

To improve the detection of *Campylobacter* spp. in retail broiler meat, a reference method (R subsamples) based on the enrichment of 25 g of meat in Bolton broth at 42 °C under microaerobiosis was compared with an alternative method (A subsamples) consisting in the rinsing of meat samples for 30 s in buffered peptone water with antimicrobials with incubation at 42 °C under aerobiosis. One piece of meat (breasts, tenderloins and thighs) was rinse in experiment 1 (A1) and two pieces in experiment 2 (A2). *Campylobacter* spp. were isolated on agar plates and identified by PCR. Retail samples in Alabama had less prevalence ($P \leq 0.05$) than samples in the state of Washington. The percentage of positive was higher ($P \leq 0.05$) in A than in R subsamples and rinsing two pieces of meat yielded the highest percentage of positive subsamples. R subsamples showed variations in the prevalence by product. However, A subsamples had similar prevalence of positives among products compare to the result from reference method. More *Campylobacter coli* isolates were collected in A2 subsamples. Pulse field gel electrophoresis (PFGE) was used as subtyping method to study the genome similarity among the isolates from all methods. A larger diversity of isolates were detected by PFGE in A2 subsamples. Denaturing gradient gel electrophoresis analysis suggested that the initial bacterial populations of the meat samples impact the final bacterial profile after enrichment. Rinsing broiler meats was less time consuming, required less sample preparation and was more sensitive than the reference method for the isolation of naturally occurring *Campylobacter* spp. This new method could help with epidemiological and intervention studies to control *Campylobacter* spp.

1. Introduction

The first isolation of *Campylobacter* spp. from commercial broiler meat in the USA was conducted in 1974 by rinsing whole carcasses in 250 ml of nutrient broth (Smith and Muldoon, 1974). This rinse methodology was used throughout the 1970s and 1980s (Fricker, 1984; Grant et al., 1980) and is still the method recommended by the Food Safety and Inspection Services of the U.S. Department of Agriculture (FSIS USDA) to test for *Campylobacter* spp. in processed poultry carcasses (Anonymous, 2009a). However, retail broiler meat is tested using a procedure developed in the 1970s for the isolation of *Salmonella* and based on the enrichment of 25 g of meat in 225 ml of broth (1:9 ratio). Samples are enriched under microaerobic conditions and the isolation of *Campylobacter* spp. is performed using selective plate media. This is the official method recommended by the Food and Drug Administration (FDA; 24), the International Organization for Standardization, the Health Protection Agency of the UK, and several regulatory agencies in other countries (Anonymous, 1998, 2006).

Many changes have occurred in the processing and selling of broiler meat products in the last 30 years and today the sale of whole chicken carcasses is minimal. Boneless, skinless broiler meat in tray packs constitutes the bulk of the current broiler meat sale in the USA. The FSIS USDA testing for the *Campylobacter* spp. performance standard is carried out after the chilling of the broiler carcasses; the last step in processing where the integrity of the whole carcass is still maintained (Anonymous, 1995; Blankenship et al., 1975). There is no performance standard for *Campylobacter* spp. for retail broiler meat.

Some publications have reviewed the different sampling techniques (Legan and Vandeven, 2003), sample preparation (Brehm-Stecher et al., 2009), and different separation and concentration protocols (Sharpe, 2003) to isolate bacterial foodborne pathogens. However, the actual sampling protocol to test retail broiler meat for *Campylobacter* spp. has not changed in the last 30 years. Therefore the current sampling method has never been challenged or evaluated to determine the most appropriate protocol for detection of *Campylobacter* spp. in skinless, boneless meat in tray packs.

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Our previous research has focused on the optimization of the methodology for isolating *Campylobacter* spp. from boneless, skinless retail broiler meat. We have shown that 1) buffered peptone water (BPW) can be used as a substitute for Bolton broth (Oyarzabal et al., 2007); 2) a 1:4 (meat:broth) ratio can be used to reduce the final enrichment volume and allow for the testing of more samples (Oyarzabal and Liu, 2010; Oyarzabal et al., 2007); 3) the removal of blood as a supplement from enrichment broths do not impact recovery (Liu et al., 2009); 4) the incorporation of filter membranes reduce competing bacteria and limit the use of antimicrobials in agar plates (Speegle et al., 2009; Williams and Oyarzabal, 2012); and 5) enrichment broths incubated under aerobic conditions generate the microaerobiosis necessary to isolate naturally-occurring *Campylobacter* spp. (Zhou et al., 2011). In addition, we have reported that the enrichment of 25 g of meat is not the optimal sample methodology to test boneless, skinless poultry meat in tray pack and can result in false negative samples (Liu et al., 2009; Oyarzabal et al., 2007). Retail broiler meat makes up more than 90% of the current retail broiler meat products sold in the USA. Therefore, an optimized methodology would allow us to determine the true prevalence of *Campylobacter* spp. and help us make correct food safety decisions in the future.

We undertook this research to assess the ability of a rinse method based on BPW to enrich retail broiler meat to isolate *Campylobacter* spp. We hypothesized that an increase in surface area would increase the isolating rate of *Campylobacter* spp. from retail broiler meat. We compared this new rinse method with a reference methodology that employs Bolton broth for the enrichment of the samples. Isolation of *Campylobacter* colonies and confirmation of presumptive isolates were performed in the same fashion for the reference and alternative method. Our simplified rinse method confirmed again that the reference methodology is not optimized for the best recovery of *Campylobacter* spp. from retail broiler meat. The methodology presented here is simpler and more cost effective than reference methods, and can be easily incorporated in food microbiology laboratories to isolate *Campylobacter* spp. from retail poultry meat.

2. Methods

2.1. Collection and analysis of samples

Raw, skinless, boneless broiler meat samples were purchased from grocery stores in Montgomery and Auburn, Alabama, and Seattle, Washington. Each sample was a tray pack. Fig. 1 shows the experimental design for these studies. In each experiment (experiment 1 and experiment 2), 120 independent samples (breasts = 40, tenderloins = 40, thighs = 40) were analyzed for the presence of naturally occurring *Campylobacter* spp. From each meat package, two sub-samples were taken. In experiment 1, one subsample was the reference subsample (R1), which consisted of 25 g of meat mixed with 100 ml Bolton broth supplemented with 5% lysed horse blood, 33 mg per l of cefoperazone and 4 mg per l of amphotericin B (Oyarzabal et al., 2007; Williams and Oyarzabal, 2012). The alternative sub-sample (A1) consisted of one piece of meat that was rinsed for 30 s in 100 ml of buffered peptone water (no addition of blood) supplemented with similar concentrations of cefoperazone and amphotericin B as the reference subsamples (Williams and Oyarzabal, 2012). The meat portions were removed with sterile tweezers after rinsing and only the rinse was incubated. In experiment 2, the reference subsamples (R2) was identical to R1 but the alternative subsamples (A2) included the rinsing of two pieces of meat.

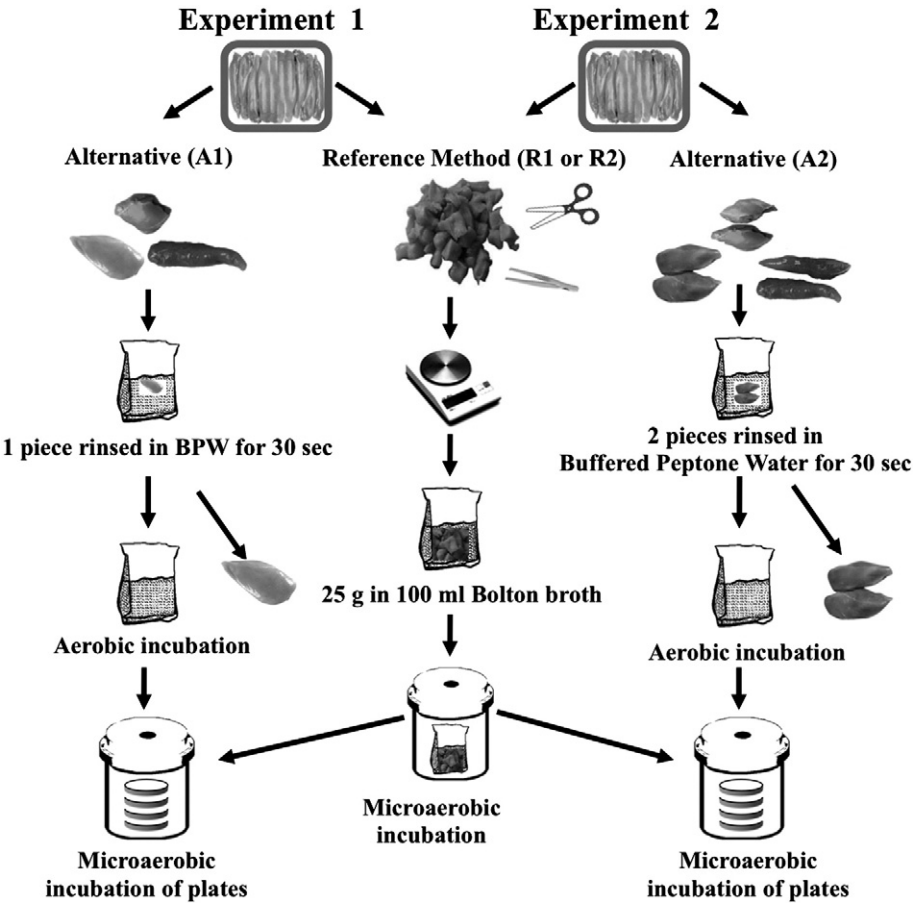

Fig. 1. Schematic representation of the alternative (rinse) methodology and the reference method used in these studies. The figure include sample preparation and isolation steps.

2.2. Isolation of Campylobacter spp.

Reference subsamples were incubated under microaerobic conditions consisting of 5% O_2, 10% CO_2 and 85% N_2 (Airgas, Radnor, PA). Alternative subsamples (A1 and A2) were incubated under aerobic conditions as described elsewhere (Zhou et al., 2011). All subsamples (reference and alternative) were incubated at 42 °C for 48 h and presumptive positives were confirmed by transferring 0.1 ml of the enriched broth onto charcoal cefoperazone deoxycholate (CCDA) agar plates using filter membranes (Speegle et al., 2009). Plates were incubated microaerobically for 48 h. Presumptive *Campylobacter* colonies were storage at −80 °C for further identification (Zhou et al., 2011).

2.3. Identification of Campylobacter isolates

Isolates were identified as *Campylobacter jejuni* or *Campylobacter coli* with a multiplex polymerase chain reaction (mPCR) assay that targets the aspartokinase gene of *C. coli* (Linton et al., 1997) and the hippuricase gene of *C. jejuni* (Persson and Olsen, 2005). This mPCR has been extensively tested for the identification of *Campylobacter* spp. isolated from retail broiler meat (Oyarzabal et al., 2007; Williams and Oyarzabal, 2012; Zhou et al., 2011).

2.4. Pulsed field gel electrophoresis (PFGE)

Selected isolates collected from both sub-samples from the same sample were typed using PFGE with the use of *Sma*I as previously described (Zhou et al., 2011). Band patterns were analyzed with BioNumerics (Applied Maths, Austin, TX) using the Dice coefficient and the unweighted pair group method with arithmetic averages (UPGMA). A 2% band optimization, 4% band tolerance and ≥90% cutting point for similarity were used in the analysis.

2.5. Denaturing gradient gel electrophoresis (DGGE)

DNA was extracted from the 48-h enriched subsamples (reference and alternative) using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). A 492 bp segment of the 16S rDNA was amplified with primers 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) (27) containing the GC clamp, and 830R (5′-ACGGCATGGACTACCAGGG-3′). PCR was performed in 25 μl reactions with 2ul DNA template. DGGE was performed in a DCode universal mutation detection system (Bio-Rad Laboratories) with a range of urea/formamide denaturing of 40 to 60% (Zhou et al., 2011). Randomly chosen samples from preliminary experiments were selected as standard bands to normalize the gels. Gel bands were analyzed with BioNumerics using the Pearson correlation and UPGMA.

2.6. Statistical analysis

We used a paired sample design in which two sub-samples from the same sample were enriched under two different conditions: the reference method (R1 or R2) and the alternative method (A1 or A2). The data were not assumed to have a normal distribution. The use of broiler meat samples naturally contaminated with *Campylobacter* spp. guaranteed that the comparison was accomplished with samples contaminated at the limit of detection of these methods (Brunelle, 2008). Results were analyzed with the McNemar's chi-squared (χ^2) test for count data (Fay, 2011; McNemar, 1947). The proportion of positives by product was analyzed with the Fisher's Exact Test for count data and the performance indicators for qualitative methods were calculated from 2-by-2 tables according to Hanrahan and Madupu (1994). All the statistical analyses were performed with R (Anonymous, 2011a).

3. Results

The average weight of the tray pack samples and the meat pieces or portions that were tested in these experiments are shown in Table 1. Table 2 shows the number of samples that were positive for *Campylobacter* spp. per experiment and per product (breasts, tenderloins or thighs), and the McNemar test results for the comparison between the reference and the alternative methods for experiment 1 and experiment 2 and for each product. When combining the results from all products by experiment, the alternative subsamples A1 yielded 21 (18%) more positives than the reference subsamples, and alternative subsamples A2 yielded 56 (47%) more positives than reference subsamples ($P \leq 0.01$). In experiment 1, there were no differences in the number of positive subsamples between the reference method and the alternative method for thighs (Table 2).

Table 3 shows the prevalence of *Campylobacter* spp. per experiment when the number of positive samples was analyzed based on region, Alabama (Montgomery and Auburn) and Washington (Seattle). A higher prevalence was found in samples from the state of Washington. R subsamples showed larger variations in the prevalence by product (percentage of positive samples by product) than the alternative subsamples, which had a closer prevalence of positives among the three products and therefore yield more consistent results. The processing plants from where these products originated are located in the states of GA, MS, NC, TN and VA, for the samples tested in Alabama, and the states of CA and WA for the samples tested in Washington. Fig. 2 shows the boxplot distribution of the percentage positives for *Campylobacter* spp. when the three products were combined and analyzed by experiment.

With the reference method, thigh samples had a higher but non-statistically significant prevalence of *Campylobacter* spp. than breast ($P = 0.26$; 95% CI = 0.20–1.44) and tenderloins ($P = 0.11$; 95% CI = 0.15–1.18). In two samples from experiment 1 and one sample from experiment 2, the reference subsample had one *Campylobacter* spp., either *C. coli* or *C. jejuni*, while the alternative subsample had the other species. The performance indicators for alternative methods vs. the reference method showed an increase in the negative predictive values (0.78 for experiment 1 and 0.94 for experiment 2) and the false positive rate (0.45 for experiment 1 and 0.78 for experiment 2). On the other hand, there was a decrease in the positive predictive values (0.54 for experiment 1 and 0.45 for experiment 2) and the false negative rate (0.22 for experiment 1 and 0.02 for experiment 2). For A1 subsamples the accuracy was 0.64 and the specificity was 0.55. For A2 subsamples the accuracy was 0.52 and the specificity was 0.22.

Table 4 shows the percentage of *C. coli* and *C. jejuni* by each experiment. The ratio of *C. jejuni* vs. *C. coli* isolates from A1 subsamples was similar to the ratio found for R1 subsamples. However, a different ratio of *C. jejuni*:*C. coli*, where more *C. coli* strains were detected, was found in A2 subsamples vs. R2 subsamples.

PFGE profiles were more diverse, corresponding to the larger number of strains isolated, for A2 subsamples. There were 38 samples for experiment 1 and 46 samples for experiment 2 in which both subsamples (reference and alternative) were positive for *Campylobacter* spp. Pair-comparisons of isolates representing 19 samples from experiment 1

Table 1
Average weight of the tray packages and the pieces (1 or 2) tested in these experiments.

	Experiment 1		Experiment 2	
	Package weight (g)	Portion weight (g)	Package weight (g)	Portion weight (g)
Average	675	162	703	285
Std Dev	167	62	154	135
Ratio of Meat:BPW (g:ml) [a]		1.6:1		2.8:1

[a] Ratio of Meat:BPW (g:ml) = ratio of the weight of meat in g in 100 ml of buffered peptone water (BPW).

Table 2
Number of *Campylobacter* positive samples analyzed by the reference (R1 and R2) and the alternative methods (A1 and A2). The analysis was performed with the exact McNemar test for count data (Fay, 2011). Calculations were performed with R. Statistical significance was set at $P \leq 0.05$.

	Number of positive (%)					
	Experiment 1			Experiment 2		
Product	R1	A1	*P*-value	R2	A2	*P* value
Breast	15 (38)	24 (60)	0.035	13 (33)	33 (83)	1×10^{-5}
Tenderloin	13 (33)	22 (55)	0.003	15 (38)	35 (88)	1.9×10^{-6}
Thigh	21 (53)	24 (60)	0.647	19 (48)	35 (88)	3×10^{-5}
Total	49 (41)	70 (58)	0.001	47 (39)	103 (86)	4×10^{-16}

and 22 samples from experiment 2 were done using PFGE to type each isolate. Results from experiment 1 showed that for 37% of the samples (7 samples), in which *Campylobacter* isolates were obtained from subsamples R1 and A1, the isolates had a DNA similarity of ≥90% between themselves (Fig. 3). A similar comparison for experiment 2 showed that for 45% of the samples (10 samples), in which *Campylobacter* were obtained from subsamples A2 and R2, the isolates had a DNA similarity of ≥90% between themselves (Fig. 4).

DGGE analysis showed a large variability in the DNA profile between R and A subsamples for both experiments. The number of bands obtained per subsample in DGGE studies varied from 2 to 12. No correlations were found between the DGGE patters of the R and A subsamples for both experiments (data not shown).

4. Discussion

In previous studies, we found that *Campylobacter* spp. are approximately at 0.6–0.8 CFU per g of retail meat. However, 6% of the samples had 1 to 5 CFU per g and approximately 2% of the samples were contaminated with more than 10 CFU per g of meat (Oyarzabal et al., 2007). The alternative methods described here were conceived with these values in mind and assuming that the rinsing of one or two pieces of meat would increase the surface area and would in turn increase the probability that a sample would be detected as positive if it were contaminated with low numbers of *Campylobacter* spp. Our results confirmed this hypothesis. The second alternative methods, where two pieces of meat were rinsed, resulted in the highest prevalence of *Campylobacter* for the tray pack products that were sampled. This highest prevalence may be the closest approximation to the actual number of positive samples in retail products, although we do not have a point of reference for comparison purposes.

In the present study, the prevalence with the alternative methods was higher than the reference method, especially when rinsing two pieces of meat. Therefore, the current reference methodology underestimates the actual prevalence of *Campylobacter* spp. in retail broiler meat, even when an increased number of positive samples is reported by combining the results of two or more 25-g subsamples (Liu et al., 2009; Oyarzabal et al., 2007). To date, the improvements to the

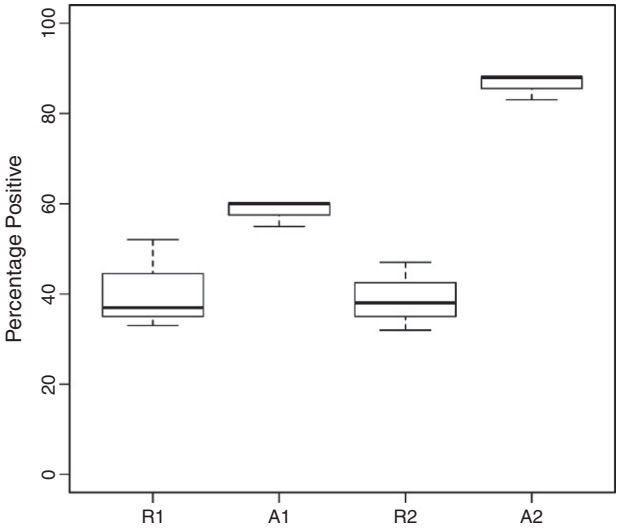

Fig. 2. Boxplot diagram of the percentage positive by the reference and alternative methods for experiment 1 and experiment 2. The graph was created with R (Anonymous, 2011a).

isolation protocols have included the use of a filter membrane, a reduction in the number of antimicrobials incorporated in enrichment and plate media, and the aerobic enrichment of the sample. Yet, the isolation prevalence has been still similar to the reference method and the main gains have related to yielding pure *Campylobacter* colonies on plates and having a simplified enrichment protocol (Speegle et al., 2009; Zhou et al., 2011).

A limitation of the current methodologies for the isolation of *Campylobacter* spp. from retail broiler meat is the 48-h enrichment time. Attempts to reduce this time to 24 h have not been successful in reducing the number of false negatives (Liu et al., 2009; Oyarzabal et al., 2007), confirming that the actual number of *Campylobacter* cell in retail broiler meat is low (Oyarzabal et al., 2007). A 48-h incubation of enrichment media appears to be better than 24 or 72 h enrichment (Fricker, 1984), and there is no scientific evidence suggesting that pre-enrichment in non-selective broth will significantly increase the number of positive samples.

Results from the nationwide microbiological baseline data collection program of FSIS USDA have shown a prevalence of 40.23% *Campylobacter* positive in processed, post-chill carcasses (Anonymous, 2009a) when using a combination of enumeration on agar plates (1.2 ml of the rinse) and the enrichment of 30 ml of the carcasses rinse (Anonymous, 2009b). But because less than 10% of the rinse sample is enriched (30 ml out of 400 ml), there is no complete understanding of the actual prevalence in processed carcasses. Unfortunately, less is known about the prevalence of *Campylobacter* spp. in retail broiler meat, which has been shown to vary extensively from country to country (Bohaychuk et al., 2006; Jørgensen et al., 2002; Kramer et al., 2000;

Table 3
Prevalence of *Campylobacter* spp. by region (Alabama and Washington) and by product. The analysis was performed by comparing the proportion of positives by product between reference and alternative subsamples with the Fisher's Exact Test. Calculations were performed with R. Statistical significance was set at $P \leq 0.05$.

		Number of positive (%)					
		Experiment 1			Experiment 2		
State	Product	R1	A1	*P* value	R2	A2	*P* value
Alabama	Breasts	10 (33)	16 (53)	0.006	8 (27)	23 (77)	1.3×10^{-12}
	Tenderloins	6 (20)	13 (43)	7×10^{-4}	8 (27)	25 (83)	8.2×10^{-16}
	Thighs	15 (50)	17 (57)	0.395[a]	13 (43)	26 (87)	5.9×10^{-11}
Washington	Breasts	5 (50)	8 (80)	1.3×10^{-5}	5 (50)	10 (100)	2.2×10^{-16}
	Tenderloins	7 (70)	9 (90)	6.5×10^{-4}	7 (70)	10 (100)	1.4×10^{-10}
	Thighs	6 (60)	7 (70)	0.181	6 (60)	9 (90)	1.2×10^{-6}

Table 4
Number and percentage of *C. jejuni* and *C. coli* by the reference (R1 and R2) and the alternative (A1 and A2) methods.

	Prevalence			
	Experiment 1		Experiment 2	
Species	R1 (%)	A1 (%)	R2 (%)	A2 (%)
C. jejuni	47 (96)	64 (91)	43 (91)	87 (84)
C. coli	2 (4)	6 (9)	4 (9)	16 (16)
Total	49	70	47	103

Madden et al., 2011; Mateo et al., 2005; Suzuki and Yamamoto, 2009; Wong et al., 2007). For instance, 25% prevalence has been reported for Switzerland (Ledergerber et al., 2003) while 100% has been reported for the Czech Republic (Hochel et al., 2004). By using a method similar to the reference protocol, the prevalence of *Campylobacter* in the USA compares equally to the 51, 58 and 66% reported in Italy, Germany and Barbados, respectively (Sammarco et al., 2010; Scherer et al., 2006; Workman et al., 2005). These results suggest that the number of CFU per gram of meat may be higher in some countries and in those instances the enrichment of 25 g may provide an appropriate methodology for detection of values closer to the true prevalence. This is probably the case with the data from Germany, where the rinse of a chicken leg resulted in a median of 4.3 log CFU/leg of *Campylobacter* spp. (Scherer et al., 2006).

C. jejuni continues to be the most prevalent species in retail broiler meat and only *C. jejuni* and *C. coli* appear to be present in commercial broiler meat in the USA (Oyarzabal et al., 2007; Williams and Oyarzabal, 2012). *C. jejuni* also appears to be the predominant *Campylobacter* spp. in retail broiler meat in most countries, except South Korea and Thailand where the predominate species is *C. coli* (Han et al., 2007; Hong et al., 2007; Meeyam et al., 2004; Padungtod and Kaneene, 2005). The fact that the ratio of *C. jejuni* vs. *C. coli* isolates from A2 subsamples was different from the other sub-sample sets suggests that the current reference method may underestimate the prevalence of *C. coli*. Further isolation and identification of *Campylobacter* strains with this new protocol may provide more data on the actual prevalence of *C. coli* in retail broiler meat in different countries.

Besides detecting a larger number of positive samples, the alternative methods also showed more consistency in the results than the reference method. Using the reference method as described in this publication, a consistently larger proportion of positive samples have been reported for thighs and a lower proportion of positive sample has been reported for tenderloins (Oyarzabal and Liu, 2010; Oyarzabal et al., 2007; Suzuki and Yamamoto, 2009; Williams and Oyarzabal, 2012). However, the alternative methods, especially from experiment 2, showed a similar proportion of positive for the three

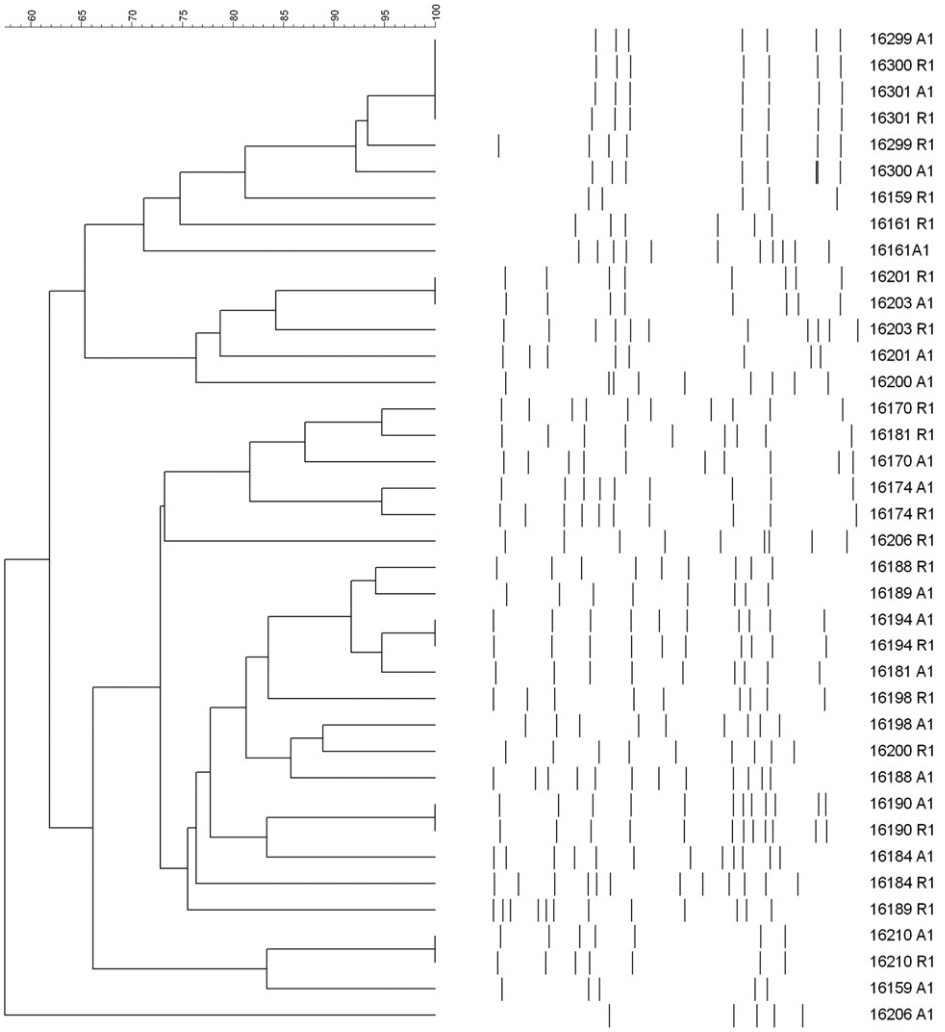

Fig. 3. PFGE profiles of *Campylobacter* isolates collected from the same sample. The figure includes isolates from reference and alternative subsamples collected in experiment 1. PFGE bands were analyzed with BioNumerics using the Dice coefficient and UPGMA.

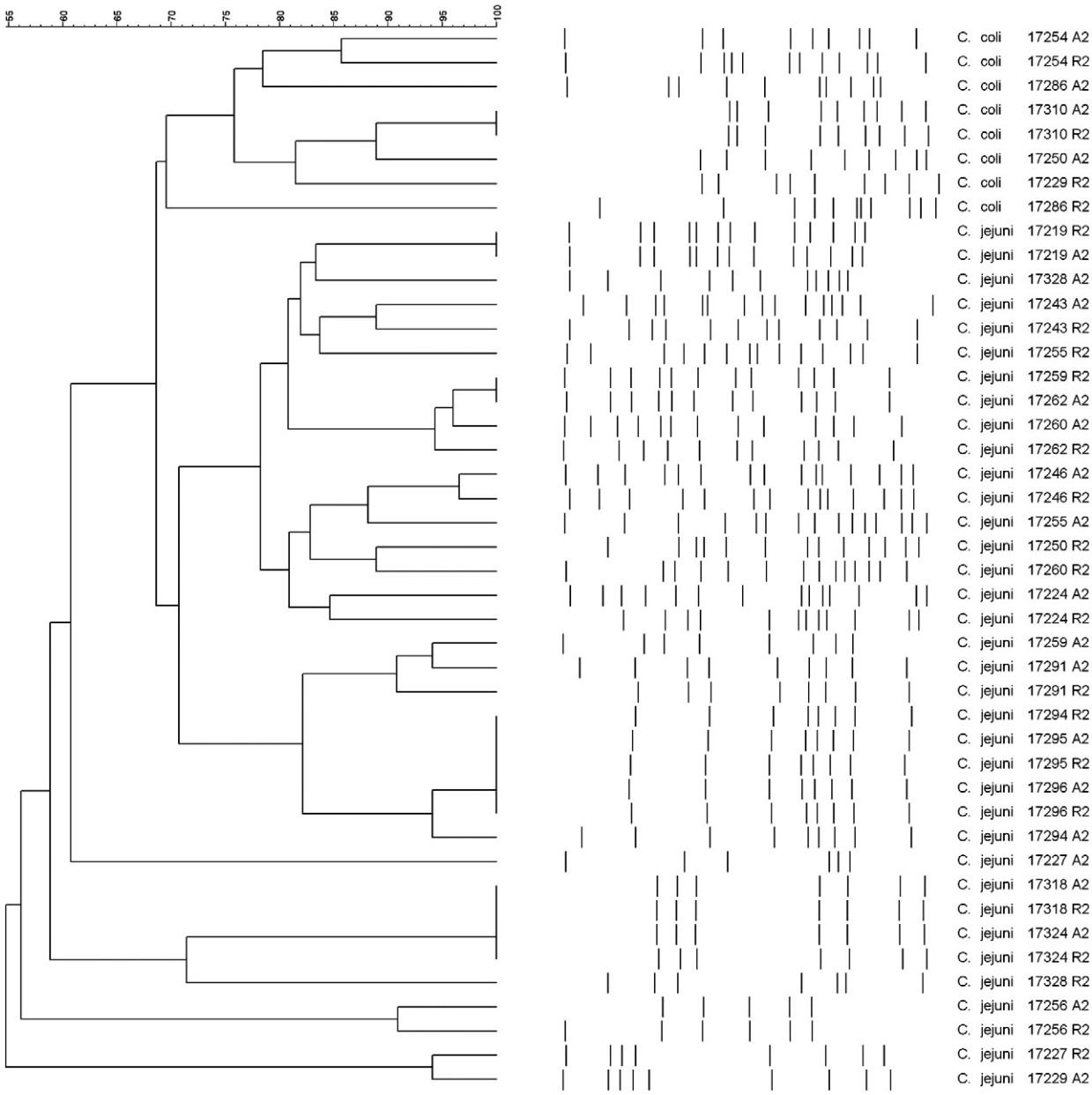

Fig. 4. PFGE profiles of *Campylobacter* isolates collected from the same sample. The figure includes isolates from reference and alternative subsamples collected in experiment 2. PFGE bands were analyzed with BioNumerics using the Dice coefficient and UPGMA.

types of products. It appears that the enrichment of only 25 g of meat may not be the most appropriate method to determine the actual prevalence of *Campylobacter* spp. in some of the commercial broiler meat products.

The statistical parameters that measured the robustness of the alternative methods (A1 and A2) vs. reference methods are considered performance indicators for quality, or count, data. In our experiments, the rinse methods were more accurate than the reference method to detect *Campylobacter* spp., and A2 subsamples had the higher negative predictive values of the two alternative methods. However, we cannot correctly use the term sensitivity and specificity because the true positivity (or negativity) of the samples is unknown. This is why the false positive rate is higher and the false negative rate is much lower in experiment 2.

If the reference method is not optimized for the detection of *Campylobacter* spp. in retail broiler meat, the term "prevalence" should be qualified to express the "most probable prevalence" with the limitations of the current reference methodology. This limitation in the way the prevalence is reported may become incorporated into the data used to calculate the national prevalence. However, our findings suggest regional variations should be taken into consideration. The verification sampling program of FSIS USDA, a risk-based testing program, tends to reward companies that pass the performance standard by placing them in Category I (Anonymous, 2011b). Once in this category, plants are sampled less frequently. However, if prevalence data is only generated from the risk-based programs, the national prevalence may be calculated only from a population of processing plants that may not be representative of the regional variations throughout the country. In this scenario, the sampling of retail broiler meat provides for prevalence data just before consumption and may help build a more significant dataset for risk assessment studies.

The nationwide raw chicken parts microbiological baseline data collection program will release their findings from testing chicken parts within the next few months (Anonymous, 2012a). This testing

has been done using 400 ml of BPW to rinse 4 lb of chicken parts, which is much less volume of rinse per amount of meat in comparison to our protocol (Anonymous, 2012b). We employed 100 ml to rinse an average of 0.35 lb in experiment 1 and 0.62 in experiment 2 (Table 1). Therefore, it will be interesting to see if this lower amount of rinse solution results in similar, lower or higher prevalence data for *Campylobacter* spp., and if there are regional variations that should be considered when analyzing these types of data.

We used PFGE to type the isolates because PFGE is one of the most robust and suitable typing methods for *Campylobacter* spp. (Behringer et al., 2011). The variability of the PFGE results found in these experiments showed that the reference method does not capture the actual variability in the PFGE profiles of the *Campylobacter* spp. contaminating retail broiler meat. Cross-contamination during processing is an important route for acquisition of *Campylobacter* strain by carcasses from *Campylobacter*-free chickens (Potturi-Venkata et al., 2007). With the increased sensitivity afforded by the alternative method, there is a tool now to track the movement of different strains across production and processing with better accuracy. There are several publications associating *C. jejuni* from chickens to diarrhea in humans, and although our current data may bias the way we associate *Campylobacter* outbreaks to poultry products in the EU and to dairy products in the US (Greig and Ravel, 2009), poultry meat continues to be a leading source of campylobacteriosis worldwide (Black et al., 2006; Stafford et al., 2008).

The analysis of DGGE bands yielded results similar to our previous observations using primers that amplify a shorter segment of the 16S DNA (Zhou et al., 2011). The present DGGE results showed again that few main bacterial populations make up the contaminants that grow under microaerobic conditions at 42 °C, and that incubation under aerobic or microaerobic environment do not play a major role on the final microbial population after enrichment because facultative anaerobes and microaerobic bacteria grow in broth enriched under aerobic or microaerobic conditions (Zhou et al., 2011). It appears that the initial contamination of the meat sample determines the final bacterial profile after enrichment. However, further studies with metagenomics and pyrosequencing analysis are needed to elucidate the key bacterial populations that grow in these products during enrichment. These studies could help in the development of more efficient methods for the rapid isolation of *Campylobacter* spp.

Currently, few laboratories test for *Campylobacter* spp. and these pathogens are the ones most likely to be missed in microbiological testing. Reports from proficiency tests have shown that the average false-negative results for *Campylobacter* spp. tests for the years 1999–2007 was 13.6%. When *C. coli* was used in proficiency tests, 24.0% of the laboratories failed to detect this pathogen (Edson et al., 2009). The alternative methods described here provides for a simplified protocol where there is no need for extensive manipulation of the sample (i.e., cutting and weighing), or the incubation of the enrichment broth under microaerobiosis. With the present study, we go a step further in the simplification of the sample preparation while increasing the efficiency of detection.

Because of the widespread contamination of raw broiler meat with *Campylobacter* spp., more interventions should be studied to produce less contaminated chicken meat. This new method may also help with experimental protocols aimed at studying interventions to reduce the number of *Campylobacter* positive samples in retail broiler meat, or for the testing of chicken parts after the application of antimicrobial treatments by poultry processors.

In conclusion, these results showed that the alternative enrichment method is a suitable enrichment protocol for the recovery of *Campylobacter* spp. from retail broiler meat under aerobic incubation. The rinsing of meat parts in BPW is less time consuming, requires less sample preparation, is less expensive than the reference method and provides for a more efficient detection of the actual number of positive samples. This new methodology may provide a better sensitivity for source attribution and intervention studies aimed at understanding the epidemiology and reducing the prevalence of *Campylobacter* spp. in retail broiler meat.

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