

Research Note

Efficacy of Mini VIDAS for the Detection of *Campylobacter* spp. from Retail Broiler Meat Enriched in Bolton Broth, with or without the Supplementation of Blood

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MS 09-265: Received 15 June 2009/Accepted 27 July 2009

ABSTRACT

The goals of this study were to evaluate the efficacy of the mini VIDAS automated immunoassay chemistry system to detect *Campylobacter* spp. from retail broiler meat enriched in Bolton broth supplemented with lysed blood (B+B) or without blood (B-B), and to detect positive samples at 24 versus 48 h after enrichment. Retail broiler meat was enriched and tested for *Campylobacter* spp. with the mini VIDAS and with an agar plate. Isolates were speciated with a multiplex PCR and typed with pulsed-field gel electrophoresis (PFGE) to evaluate relatedness of isolates collected from subsamples enriched in B+B or B-B. The number of *Campylobacter*-positive samples by mini VIDAS was similar ($P > 0.05$) to the results found with traditional plating media for naturally contaminated broiler meat, regardless of whether the comparison was made between B+B and B-B, or among different meat products (breast, tenders, and thighs). More positive samples were found at 48 h of enrichment than at 24 h of enrichment ($P < 0.05$). A *Campylobacter jejuni*:*Campylobacter coli* ratio of 4:1 was found in this study. Most of the isolates from both subsamples (B+B and B-B) were similar or identical by PFGE analysis, except for a few samples in which the PFGE profiles of the isolates from the subsamples were different. Mini VIDAS allowed for the detection of *Campylobacter* spp. within 48 h after enrichment. However, the sensitivity is similar to plate media, and retail broiler samples need to be enriched for 48 h to avoid false negatives.

Campylobacter spp. are common etiological agents of diarrhea worldwide. In the European Union, *Campylobacter* infections have been the most reported bacterial foodborne diseases since 2005 (4), and in the United States, the rate of human campylobacteriosis has been relatively constant since 2001 (6). Currently, there is no regulation in the United States requiring the testing of broiler meat for the presence of *Campylobacter* spp. However, if regulations are established, the food industry has few options of automated equipment for the rapid screening of samples for *Campylobacter* spp. One of these automated systems is the mini VIDAS, which is a multiparameter, automated immuno-analyzer in a compact form.

The VIDAS has been used to detect *Campylobacter* spp. in broiler carcasses enriched in Preston broth (24) and in gastrointestinal contents, lymph nodes, and tonsils of pigs enriched in Preston broth (18). Yet, there are few studies assessing the efficacy of the VIDAS method to detect *Campylobacter* spp. in retail broiler meat enriched in Bolton broth, with and without the addition of blood, and at 24 h versus 48 h of enrichment.

We have recently demonstrated that a 1:4 enrichment ratio (25 g of meat in 100 ml of broth), using buffered peptone water as the enrichment medium, compared similarly to Bolton broth or buffered peptone water at a 1:9 ratio (25 g meat in 225 ml of broth) for the detection of *Campylobacter* spp. from retail broiler meat (19). To provide some practical information about the use of mini VIDAS to detect *Campylobacter* spp., we assessed the efficacy of mini VIDAS for the detection of *Campylobacter* spp. from retail broiler meat enriched in Bolton broth with or without the addition of lysed horse blood. Experiments were done with an enrichment ration of 1:4. A comparison of the detection rate of mini VIDAS at 24 and 48 h was also performed. The isolation of *Campylobacter* spp. from the same samples on modified Campy-Cefex (mCC) plates was used as the reference method for comparison purposes. Isolates were identified to the species level with a multiplex PCR assay (mPCR) and were typed with pulsed-field gel electrophoresis (PFGE) to compare the restriction profiles of isolates collected from the same samples.

MATERIALS AND METHODS

Media and cultural conditions. Ninety-three retail broiler meat samples (43 skinless, boneless breasts; 21 tenders; and 29 thighs) were purchased from local food stores from 16 July 2008 through 22 May 2009. The meat was cut into pieces approximately

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TABLE 1. Results of testing meat samples enriched in B+B or B-B for the presence of *Campylobacter* spp.^a

Product	No. of samples	Positive samples				True positives ^d
		B+B ^b		B-B ^c		
		Mini VIDAS	mCC	Mini VIDAS	mCC	
Breast	30	18	16	22	21	25
Tenders	11	1	0	1	1	2
Thighs	14	8	7	9	8	9
Total	55	27	23	32	30	36

^a The comparison was done between the results of testing the samples at 48 h with mini VIDAS and mCC plates. A sample was considered true positive if a positive result was found for at least one of the subsamples (B+B or B-B) by mini VIDAS or mCC at 48 h.

^b $\chi^2 = 3.1$ ($P = 0.57$), sensitivity = 0.81, specificity = 0.91, kappa = 0.71, false-positive rate = 0.09, false-negative rate = 0.19, positive predictive value = 0.93, accuracy = 0.85. The kappa value is a quantitative measure of the magnitude of agreement between observers, or an inter-rater agreement, for qualitative (categorical) observations (27). The accuracy of the test is defined as the proportion of true results among all test results (15).

^c $\chi^2 = 2.3$ ($P = 0.31$), sensitivity = 0.88, specificity = 0.97, kappa = 0.85, false-positive rate = 0.03, false-negative rate = 0.12, positive predictive value = 0.96, accuracy = 0.93.

^d Comparison between positive from B+B and true positives: $\chi^2 = 7.1$ ($P = 0.002$), kappa = 0.67, false-positive rate = 0.25, positive predictive values = 0.68, accuracy = 0.84. Comparison between B-B and true positives: $\chi^2 = 2.3$ ($P = 0.051$), kappa = 0.85, false-positive rate = 0.11, positive predictive value = 0.83, accuracy = 0.93.

1 in² (6.45 cm²), and then it was divided into two subsamples of 25 g each. Subsamples were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI). One subsample was enriched in Bolton broth (Acumedia, Lansing, MI) with the addition of selective supplements (Oxoid, Inc., New York, NY) and 5% (vol/vol) lysed horse blood (B+B). The other subsample was enriched in Bolton broth with the addition of the selective supplement but without the addition of blood (B-B). The enrichment ratio was 1:4 (25 g of meat in 100 ml of the enrichment broth). Samples were enriched at 42°C for up to 48 h under microaerobic conditions (10% CO₂, 5% O₂, and 85% N₂; Airgas, Radnor, PA) generated by a MACSmics Jar Gassing System (Microbiology International, Frederick, MD) in anaerobic jars.

Evaluation of mini VIDAS. An evaluation between detection of *Campylobacter* spp. by conventional plate media and the mini VIDAS automated immunoassay chemistry system (bioMérieux, Inc., Hazelwood, MO) was performed in both subsamples from 55 samples (Table 1). Enriched broth (0.1 ml) was passed through a cellulose membrane filter of 0.65- μ m pore size (Millipore Corp., Billerica, MA), which was placed onto mCC agar plates (21). Plates were incubated at 42°C under microaerobic conditions for 48 h. Presumptive *Campylobacter* isolates on plates (spiral shape and darting motility under phase contrast microscopy) were collected and stored at -80°C in Brucella broth (Acumedia) supplemented with 30% glycerol (vol/vol) and 5% blood for further identification and characterization.

Evaluation of 24 h versus 48 h of enrichment. We analyzed the *Campylobacter*-positive rate at 24 versus 48 h in 50 B+B subsamples and in 57 B-B subsamples by using only plate media (Table 2). Isolates on agar plates were presumptively identified as *Campylobacter* spp. under phase contrast microscopy. In addition, a comparison of the detection rate between mini VIDAS and mCC plates at 24 versus 48 h of enrichment was performed with 19 B+B subsamples and 7 B-B subsamples.

Identification of isolates. Fifty-six isolates (Table 1) were grown on blood agar plates from -80°C stocks, and DNA was

extracted with PrepMan Ultra (Applied Biosystems, Foster City, CA). An mPCR assay that detects *Campylobacter jejuni* and *Campylobacter coli* was performed in 25- μ l aliquots, as described elsewhere (17, 19, 22).

PFGE analysis. A standard PFGE protocol with a few modifications described elsewhere (20) was used to determine the genomic variability of *Campylobacter* isolates collected from different subsamples (B+B and B-B) from the same sample. *Salmonella choleraesuis* subsp. *choleraesuis* serotype Braenderup H9812 (ATCC BAA-664) restricted with *Xba*I was used as the DNA size marker (3). Pair comparisons and cluster analyses were performed with the Dice correlation coefficient and the unweighted pair group mathematical average clustering algorithm. The position tolerance for band analysis was set at 3%, and a cutoff of 90% DNA relatedness was used to determine if isolates were similar.

Statistical analysis. Results from mini VIDAS and mCC plates at 48 h were analyzed with the chi-square (χ^2) test according to McNemar, wherein $\chi^2 \leq 3.84$ indicated that the results from the test method (mini VIDAS) and the reference method (mCC plates) are equivalent ($P > 0.05$). A sample was considered true positive if a positive result was found for at least one of the subsamples (B+B or B-B) by mini VIDAS or mCC. The chi-square test was also used to compare the values at 24 versus 48 h. The χ^2 and kappa values were obtained with PROC FREQ (SAS 9.1, SAS Institute, Inc., Cary, NC). The sensitivity, specificity, false-positive rate, false-negative rate, and the accuracy of mini VIDAS were calculated with the 2-by-2 tables generated by SAS and according to Hanrahan and Madupu (15).

RESULTS AND DISCUSSION

The number of *Campylobacter*-positive samples by mini VIDAS was similar ($P > 0.05$) to the results found with traditional plating media for the isolation of *Campylobacter* spp. for naturally contaminated broiler meat (Table 1), regardless of whether the comparison was made between B+B and B-B samples, or among different meat products (breast, tenders, and thighs). This automated

TABLE 2. Results of testing meat subsamples enriched in B+B or B-B for the presence of *Campylobacter* spp.^a

Enrichment	Product	No. of samples	Positive samples	
			24-h incubation	48-h incubation ^b
B+B	Breast	22	2	12
	Tenders	18	1	4
	Thighs	17	4	7
	Total ^c	57	7	23
B-B	Breast	20	3	13
	Tenders	15	1	5
	Thighs	15	2	6
	Total ^d	50	6	24

^a The comparison was made between positive samples at 24 h versus total positive samples at 48 h. Samples were considered positive if *Campylobacter* isolates were detected on mCC plates at 24 or 48 h.

^b Forty-eight-hour positives include 24-h positives.

^c $\chi^2 = 14.1$ ($P < 0.001$), kappa = 0.34, false-positive rate = 0.70, positive predictive values = 0.68, accuracy = 0.72.

^d $\chi^2 = 16.1$ ($P < 0.0001$), kappa = 0.26, false-positive rate = 0.75, positive predictive values = 0.59, accuracy = 0.64.

immunoanalyzer system performed similarly to conventional plating media, but had the advantage that samples can be screened for positive or negative results in approximately 4 h, and resources can be used more efficiently to confirm presumptive positives. In Norway, the VIDAS CAM method has been used for the detection of *Campylobacter* spp. from tissue samples (tonsils and lymph nodes) and fecal material from pigs during processing (18). In another study involving the collection of samples from a commercial poultry processing plant located in southern Brazil, mini VIDAS was used to test for the presence of *Campylobacter* spp. in different samples, including broiler chilled carcasses and carcass parts (24). In that study, a low prevalence of *Campylobacter* spp. was reported for chilled carcasses (20%) and for chicken parts (6.7%). However, samples were enriched in Preston broth at 30°C for 24 h only. In our study, the enrichment broths were incubated for a total of 48 h, as indicated by the VIDAS CAM method, which may account for the higher number of *Campylobacter*-positive samples.

The number of positive samples (mini VIDAS plus mCC results) was 52% ($n = 29$) for B+B, 60% ($n = 33$) for B-B, and 65% ($n = 36$) were true positives (Table 1). In general, B+B yielded a lower number of *Campylobacter*-positive samples than B-B yielded, although these differences were not significant ($P > 0.05$). However, when comparing the number of *Campylobacter*-positive samples between B+B and B-B, with true positives (any positive result found for at least one of the subsamples by mini VIDAS or mCC at 48 h), B+B yielded a lower number of *Campylobacter*-positive samples ($P < 0.05$). Blood and other ingredients have been traditionally added to the formulation of *Campylobacter* media to neutralize the toxic effects of compounds produced in the presence of

oxygen and light (11). Blood is more effective than charcoal, sodium pyruvate, ferrous sulfate, and sodium metabisulfite in neutralizing hydrogen peroxide (9, 10), and lysed horse blood was the best supplement that facilitated aerotolerance among 22 supplements tested (8). However, blood has a short shelf life, is contaminated easily, and expensive. Therefore, if blood can be avoided, the overall methodology for *Campylobacter* isolation will be simplified.

The analysis of the samples enriched in B-B resulted in higher kappa and accuracy values, and lower false-positive and false-negative rates than samples did enriched in B+B. These findings suggest that the addition of blood may not be necessary for the isolation of naturally occurring *Campylobacter* spp. in retail boiler samples. Some components of blood, primarily heme, hemoglobin, and lactoferrin, have also been found to be inhibitory in the PCR assays used for identification of bacterial pathogens in biological samples (1, 2). Because molecular techniques, such as the enzyme-linked immunosorbent assay and PCR, are indispensable for an accurate identification of *Campylobacter* isolates to the species level, enriching samples in B-B may allow for the use of these molecular techniques for faster identification of *Campylobacter* spp. from enriched retail broiler meat.

A sample broth ratio of 1:4 (meat:broth [wt/vol]) has been already validated for the isolation of *Campylobacter* from retail broiler meat (19). Similar results have been found for the isolation of *Listeria monocytogenes*, where enrichment ratios of 1:3, 1:5, and 1:10 exhibited comparable growth rates (29). Our results suggest that a 1:4 ratio of B-B is comparable to a 1:4 ratio of B+B for the isolation of *Campylobacter* spp.

The enrichment of the samples for 24 h resulted in a lower number of *Campylobacter* positives compared with 48 h of enrichment ($P < 0.05$), regardless of whether the meat was enriched in B+B or B-B (Table 1). This difference was also present when the results from mini VIDAS and/or mCC plates were pooled and used for the comparison (data not shown). The comparison of the positive rates between 24 and 48 h of enrichment clearly indicated that 24 h of enrichment results in several false-negative samples. Although the accuracy was relatively low for this comparison due to the small number of samples tested, these results clearly showed that 24 h of enrichment is not sufficient for the isolation of *Campylobacter* spp. from retail broiler meat. An enrichment time of 44 ± 4 h has been suggested for the enrichment of food stuffs in Bolton broth by normative International Organization for Standardization standard 10272-1:2006 (5), and this enrichment time may be a necessity to avoid false-negative samples from retail broiler meat, which is usually contaminated with very low numbers of *Campylobacter* cells (19).

Based on true positives, the overall prevalence of *Campylobacter* spp. was 62% (58 positive samples of 93 samples). This prevalence was similar to the prevalence reported in studies from the United States (12, 28), Australia (87.8 and 93.2% (23)), Belgium (72% (14)), Japan (80% (25)), the United Kingdom (79% (16)), and other European countries (7, 13, 26). The prevalence for breast meat was

Dice (Copt:1.00%) (Tot:3.0%-3.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
All

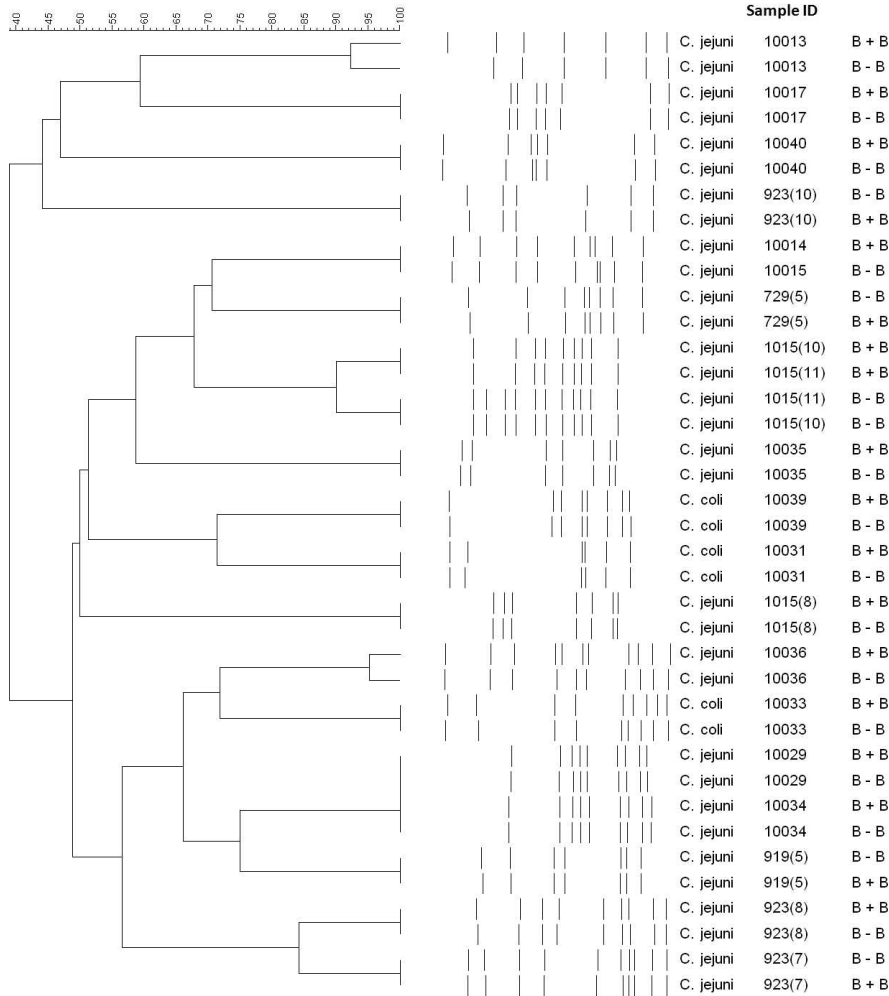


FIGURE 1. Analysis of macrorestriction profiles obtained by PFGE of *Campylobacter* isolates from samples with the same or similar isolates from each subsample. Pair comparison and cluster analysis were performed with the Dice correlation coefficient and the unweighted pair group mathematical average clustering algorithm, respectively, with BioNumerics version 4.50 (Applied Maths, Austin, TX). The position tolerance for band analysis was set at 3%. A cutoff of 90% of DNA relatedness based on band patterns was used to determine if two restriction patterns were similar (>90%) or different (<90%). B+B, Bolton with blood; B-B, Bolton without blood.

84%, 52% for thighs, and 33% for tenders. Chi-square analysis of the actual positive samples per product resulted in $\chi^2 = 17.23$ due to a lower *Campylobacter* prevalence for thighs and tenders in comparison to breast meat ($P < 0.05$).

Fifty-six isolates (26 from B+B subsamples and 30 from B-B subsamples) were identified to the species level by an mPCR assay. Forty-five (80%) isolates were identified as *C. jejuni*, and 11 (20%) as *C. coli* (*C. jejuni*:*C. coli* ratio of 4:1). In

10 samples, only one subsample was positive. Three samples yielded mixed cultures (*C. coli* and *C. jejuni*) after enrichment and from both subsamples. These cultures were purified by transferring them through 0.65- μ m-pore-size cellulose filters that were placed on top of mCC plates. Isolated colonies from these samples were then retested with mPCR, and were confirmed as *C. jejuni* for the 3 samples. We have previously reported that 7% of broiler retail samples tested with Bolton

Dice (Copt:1.00%) (Tot:3.0%-3.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
All

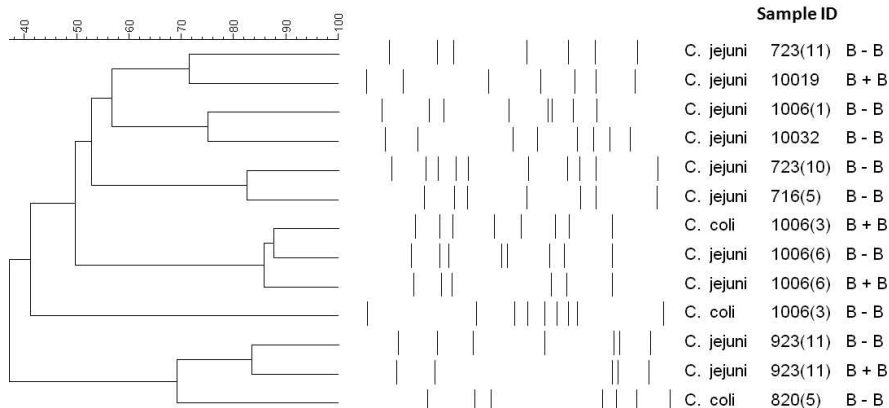


FIGURE 2. Analysis of macrorestriction profiles obtained by PFGE of *Campylobacter* isolates from samples containing different isolates in each subsample or samples having only one isolate from one subsample. Pair comparison and cluster analysis were performed as described in Figure 1. B+B, Bolton with blood; B-B, Bolton without blood.

broth and buffered peptone water had both *C. coli* and *C. jejuni* (19). It is apparent that *C. coli* and *C. jejuni* contaminating the same broiler sample can replicate in Bolton broth, without an apparent competition between each other.

PFGE analysis of isolates from the same samples but different subsamples (B+B and B-B) showed that most of the isolates from both subsamples were similar or identical (Fig. 1). However, in a few samples, only one subsample was positive for *Campylobacter*, while for other samples, the isolates from the subsamples showed less relatedness or a higher PFGE diversity (Fig. 2).

To conclude, these results suggest that mini VIDAS can shorten the time for detection of *Campylobacter* spp. in retail broiler meat, and that the addition of blood may not be necessary for the isolation of *Campylobacter* spp. from these food samples.

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