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Detection of *Campylobacter* on the Outer Surface of Retail Broiler Chicken Meat Packages and on Product Within

ABSTRACT

The objective of this study was to compare prevalence of *Campylobacter* on the outside of broiler meat packages to that on the product inside the same packages. Chicken meat products were purchased at retail. Samples comprised whole carcasses and six different cut-up-part products. Fifteen packages of each type of product were purchased (n = 105). The exterior surface of each package was sampled, the package was sanitized, and opened, and exudate or product rinse was collected. Samples were cultured for the presence of *Campylobacter* spp. Overall, 41 of 105 packages (39%) had detectable numbers of *Campylobacter* associated with the product within. This included some of each type of product. One of 105 packages had detectable numbers of *Campylobacter* on the outer surface. That package was one of six characterized as leaky. *Campylobacter* isolates were subtyped using multi-locus sequence typing; 29 sequence types identified were either *C. jejuni* (n = 19) or *C. coli* (n = 10). The outer surface isolate was the same subtype as the associated exudate isolate. Although various *Campylobacter* subtypes were found on the inside of a substantial percentage of retail broiler meat packages, the outer surface of intact, non-leaky packages can be reasonably expected to be free of *Campylobacter*.

INTRODUCTION

Bacteria of the genus *Campylobacter* are human foodborne pathogens that can be found associated with chicken meat products at retail, having been reported on conventional and organic whole broiler carcasses *(5, 11, 16, 17, 22)* and cut-up parts *(3, 20, 22)* collected at retail markets. Although *Campylobacter* are often detected on the skin surface as opposed to deep muscle tissue, skin-off broiler parts are no less likely to be contaminated than skin-on parts *(2, 4)*. Indistinguishable subtypes of *Campylobacter* were detected on chicken meat and from human campylobacteriosis patients in the same geographical area *(12)*. Furthermore, some chicken *Campylobacter* isolates are antibiotic resistant,

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which could complicate treatment of human disease *(1, 9)*. Therefore, the presence of *Campylobacter* on chicken meat products presents a significant public health concern.

Packaged chicken meat is often accompanied by a liquid exudate, sometimes called "weep," which comes from the product and is contained by the packaging. Such exudate has been found to be positive for *Campylobacter (6)* and can be a very effective sample for the determination of *Campylobacter* status of packaged broiler carcasses *(18)*. *Campylobacter* in chicken exudate is a concern for consumers as it may facilitate infection, either directly or by cross contact with other surfaces or foods. Cross-contamination from *Campylobacter*-positive chicken meat has been described in published research *(13, 14)*. Even in the case of knowledgeable food handlers, cross-contamination of hands and equipment is commonly seen in cooks observed in private kitchens *(15)*.

Campylobacter have been detected on the outer packaging of broiler chicken meat for sale at retail in the UK and New Zealand *(8, 23)*. In one study of whole carcasses at retail, 3% of outer packaging film and 34% of the inner surface of packaging film were *Campylobacter* positive, while 68% of the carcasses themselves were positive *(8)*. In another study, 24% of outer packaging was positive for *Campylobacter*, with numbers estimated to be as high as 2,200 cells per package *(23)*. While it may be assumed that the outside of a package is primarily contaminated due to leaking exudate, leaking packages were only slightly more likely to be positive than non-leaking packages *(23)*. Packaging material may be contaminated in the processing plant or elsewhere by a source other than exudate leaking from meat in the same package.

The objective of this study was to measure the prevalence and compare subtypes of *Campylobacter* from the outside surface and the exudate of non-leaking packaged fresh broiler meat products at retail.

MATERIALS AND METHODS

Experimental overview

Fresh broiler meat products (whole carcasses and cut-up parts) were purchased at a variety of retail markets. The exterior surfaces of all packages were swabbed. Packages were then opened and the exudate within each package was collected. Outer surface swabs and exudate samples were cultured for the presence of *Campylobacter*. The presence of *Campylobacter* associated with packaged product was compared to the prevalence on the outer surface of packages. Seven types of fresh product samples were included: 1. whole broiler carcasses, 2. breast halves, 3. boneless skinless breast halves, 4. thighs, 5. boneless skinless thighs, 6. drumsticks, and 7. wings. For each of the seven product types, three replicate market trips were conducted, with five samples each (n = 15 for each product, total number of samples = 105).

Sample collection

On each sample day, five packages of fresh broiler meat product were purchased at retail outlets. To represent a wide range of material, each package was purchased from a different store from multiple brands, with different plant numbers and sell-by dates. Sample packages were selected so as to exclude those that were wet or that appeared leaky. No package was touched with the bare hand of the collector. Samples were collected using an inverted plastic bag from a roller at the market. Each package was placed into a separate bag for scan at the store register and placed on ice for transport to the lab. All samples were held in separate plastic bags under refrigeration at 4°C overnight and cultured the following day.

Campylobacter culture

On the day of culturing, packages were re-examined for signs of exudate leakage, and any leaking packs were noted. All packages were sampled on the exterior surface using a sterile sponge (Whirl-pak 18 oz. speci-sponge, Nasco, Fort Atkinson, WI) pre-moistened with 10 ml of phosphate buffered saline (PBS). Each package was handled with a new clean pair of latex gloves. The entire surface was rubbed thoroughly with the sponge, which was put back into a sterile bag, covered with 50 ml *Campylobacter* enrichment broth (Bolton's formulation) (CEB) (Acumedia, Neogen Corp., Lansing, MI) and subjected to 30 s in a paddle blender (Stomacher 80, Seward, Port St. Lucie, FL). CO_2 Sponges and CEB were incubated for 24 h at 42°C in re-sealable plastic bags flushed with micro-aerobic gas (5% O_2, 10% CO_2, 85% N_2).

After exterior sponge sampling, each package was sanitized with a 70% ethanol spray and wipe product and aseptically opened; exudate was removed with a sterile pipet. In cases where at least 7 ml of exudate was not present, product was removed from the package, and placed into a clean re-sealable bag; enough sterile water to assure at least 7 ml collection (50 to 100 ml) was added prior to a 60 s hand shake. Lack of exudate was noted in many samples that included absorbent pads, especially in skin-off samples. Exudate or rinse was collected with a sterile pipet; 5 ml was added to 45 ml CEB and 1.2 ml was used for direct plating onto the surface of Campy-Cefex plates (CCA) *(21)*. Plates and broth were incubated in re-sealable bags flushed with micro-aerobic gas; broth was incubated for 24 h and plates for 48 h.

Following incubation, enrichment broth was used to streak for isolation on CCA plates, which were then incubated for 48 h at 42°C in a micro-aerobic gas atmosphere. Following incubation, all plates were examined for presence of characteristic *Campylobacter* colonies, which were confirmed as thermophilic *Campylobacter* by observation of cellular morphology and motility under phase contrast microscopy and by a positive reaction to a latex agglutination test (Microgen Bioproducts Ltd. Camberly, UK).

Campylobacter isolate characterization

One typical *Campylobacter* colony from each positive sample was selected for further characterization. Each isolate was re-streaked onto CCA to produce a lawn and grown for 24 h at 42°C in a microaerobic atmosphere, as previously described. Bacterial growth was removed from plates with a sterile cotton-tipped applicator and added to 1 ml of sterile freezing medium (blood-and-additive-free CEB with 15% glycerol) in a freezing vial (Cryovial, Simport, Beloeil, QC, Canada). Cultures were frozen and held at -80°C until all isolates were collected.

Isolates were revived from frozen storage by streaking for isolation onto the surface of tryptic soy agar with 5% sheep's blood (Remel, Lenexa, KS) and then incubated at 42°C for 48 h. One isolated colony was selected and streaked to produce a lawn on the surface of Brucella agar (Accumedia, Neogen Corp., Lansing, MI), 42°C, 24 h. All growth was removed from the Brucella agar and DNA was extracted using a commercial kit following manufacturer's instructions (UltraClean® microbial DNA isolation Kit, Mo Bio Laboratories Inc., Carlsbad, CA). Libraries were prepared using the Nextera XT sample preparation kit (Illumina, San Diego, CA). The genomic DNA of each isolate was sequenced using the Illumina MiSeq platform with a 2 X 250 paired end run according to manufacturer's instructions (Illumina, San Diego, CA).

Gene sequences were analyzed by aligning multi locus sequence type (MLST) results for loci as described by Dingle et al. *(7)*. Raw sequence read files for each isolate were mapped to reference sequences of each locus using Geneious Mapper (Biomatters Ltd., Aukland, NZ). The strict consensus for each mapping was trimmed to match the reference and then submitted to the website pubmlst.org/campylobacter/ *(10)* to obtain the allelic identifiers and the sequence type (ST) of the isolate.

Statistical analysis

Three replications were conducted for each sample type, each replication including 5 discrete samples (n = 15 per sample type, n = 105). Prevalence values were compared by the chi square test for independence.

RESULTS

One hundred five product samples were collected, representing a total of 18 identifiable processing plants. The six most commonly encountered plants represented 79 (75%) of the samples. *Campylobacter* were detected in at least some samples from fourteen plants. The four plants from which no positive samples originated were among the less frequently sampled, accounting for just 9 samples (8%).

Prevalence of *Campylobacter* detected on the packaging surface and in the exudate/rinse within the packages is presented in *Table 1*. *Campylobacter* were detected in exudate/rinse from some of all product types tested. When the prevalence was analyzed by the chi square test for independence, whole carcasses were seen to be significantly more likely to have detectable levels of *Campylobacter* than any of the cut-up parts ($P \leq 0.001$). Ninety-three percent of whole carcasses and 30% of cut-up parts were positive for *Campylobacter*.

Table 1. Prevalence (number positive/number sampled) of *Campylobacter* on the wrapper surface and in the exudate or rinsate[1] within packaged fresh broiler meat products purchased at retail

Sample	Package Exterior	Exudate	Rinsate	Sum
Whole carcasses	0/15	14/15	0/0	14/15
Breast halves	0/15	4/6	3/9	7/15
Boneless/skinless breast halves	0/15	1/1	2/14	3/15
Thighs	1/15*	0/4	5/11	5/15
Boneless/skinless thighs	0/15	0/0	6/15	6/15
Drumsticks	0/15	0/0	4/15	4/15
Wings or drumettes	0/15	1/7	1/8	2/15
Total	1/105	20/33	21/72	41/105

[1]exudate cultured in cases where at least 7 ml was available; otherwise, each part was rinsed in sterile PBS.
*_Campylobacter_ positive package was one of 6 wet, leaking packages.

Out of 105 packages of fresh broiler meat products, one had detectable *Campylobacter* on the outer surface. This was a package of skin-on, bone-in thigh portions. Although effort was made to secure only non-leaking packages, the exterior positive package was one of six packages found to be leaking when closely examined in the lab. When characterized by MLST sequence analysis, the isolate detected on the outer package surface was indistinguishable from the isolate recovered from the exudate of the same package. *Campylobacter* was not found on the outside surface of any other packages, regardless of whether the pack was leaking.

Some products had enough exudate to allow for direct culture without addition of more liquid. Others required the addition of sterile water and a rinse to sample the packaged meat. All whole carcasses had adequate exudate to facilitate sampling; at least some of all cut-up products were too dry to sample exudate directly. All samples of drumsticks and boneless-skinless thighs required addition of PBS and rinsing. The relationship between exudate volume and *Campylobacter* detection is presented in *Table 2*. In this analysis of all samples, there is a significant relationship between the presence of exudate and *Campylobacter* detection ($P = 0.002$). However, *Table 2* includes data from whole carcasses, of which all had adequate exudate and in 93% of which *Campylobacter* were detected. *Table 3* presents an alternate 2 X 2 table which includes data from only cut-up parts (no whole carcasses). In *Table 3*, it is shown that for cut-up parts there is no significant relationship between exudate volume and *Campylobacter* status. Parts with more than 7 ml of exudate were no more likely ($P = 0.73$) to have detectable numbers of *Campylobacter* than were parts with less exudate.

Multi-locus sequence typing results produce data that can identify *Campylobacter* as to species (e.g., *C. coli* or *C. jejuni*) and further differentiate clonal complexes and individual sequence types (ST). There were 42 positive samples, ten of which had two isolates, one from direct plating and another from enrichment, for a total of the 52 isolates subjected to MLST. Fifteen of the 52 isolates (29%) were *C. coli*; 37 (71%) were *C. jejuni. C. coli* were detected in product from 8 plants evenly distributed by date and product type. Of the 10 multiple-isolate samples, in 3 cases *C. coli* was detected in the enrichment culture while *C. jejuni* was detected by direct plating; in the other 7 multiple-isolate samples *C. jejuni* was detected by both culture methods.

Campylobacter isolates were characterized as *C. jejuni* or *C. coli* and further separated into 13 clonal complexes and 29 individual STs which are defined and numbered by Pubmlst *(17)*. Sequence type data are presented in *Table 4*. Within the 37 *C. jejuni* isolates, 19 STs were represented, 13 of which were only found once. Six STs of *C. jejuni* were

Table 2. Two by two table for prevalence of *Campylobacter* (number positive/number sampled) in exudate or rinsate[1] from all fresh broiler meat products (parts and whole carcasses) with and without at least 7 ml of exudate in the package

Exudate Present	*Campylobacter* Positive	*Campylobacter* Negative
Less than 7 ml	21	51
More than 7 ml	20	13

[1]exudate cultured in cases where at least 7 ml was available; otherwise, each part was rinsed in sterile PBS.
Significant by chi square test for independence, $P = 0.002$.

Table 3. Two by two table for prevalence of *Campylobacter* (number positive/number sampled) in exudate or rinsate[1] from fresh cut-up broiler parts with and without at least 7 ml of exudate in the package

Exudate Present	*Campylobacter* Positive	*Campylobacter* Negative
Less than 7 ml	21	51
More than 7 ml	6	12

[1]exudate cultured in cases where at least 7 ml was available; otherwise, each part was rinsed in sterile PBS.
Non-significant by chi square test for independence, $P = 0.73$.

Table 4. Multi-locus sequence type (ST) of *Campylobacter* isolates detected on packaged fresh broiler meat products

Species	Clonal Complex[1]	ST[1]	Isolates[2]	Samples[3]	Dates[4]	Plants[5]
C. jejuni	21	21	3	2	1	1
		50	1			
		982	1			
	22	22	1			
	41	41	1			
	48	48	3	3	3	1
		475	2	1		
		6639	1			
	49	467	2	1		
		6645	1			
	52	52	2	1		
	353	353	4	4	3	3
		452	3	3	2	3
		2132	1			
		3510	3	3	3	2
		3735	2	1		
	443	51	1			
	NV[6]	468	1			
	NV	1839	4	2	2	1
C. coli						
	828	825	1			
		829	2	2	2	1
		899	1			
		1017	1			
		1050	1			
		1063	1			
		1082	1			
		7818	5	5	4	3
		7827	1			
	1150	7816	1			

[1]Clonal complex and sequence type as defined and reported on PubMLST website: http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_campylobacter_isolates

[2]Number of isolates of this ST detected

[3]Number of samples in which this ST was detected

[4]Number of sample dates on which this ST was detected

[5]Number of distinct broiler processing plants from which this ST was detected

[6]NV: no value assigned by PubMLST

found in multiple samples representing different sample days, processing plants, or product types. The most prevalent ST was ST 353, which was detected in 4 different samples from 3 different plants on 3 different sample dates. Within the 15 *C. coli* isolates, 10 STs were represented, 8 of which were found only once. The most prevalent ST of *C. coli* was ST 7818, which was found in 5 separate samples from 3 different processing plants on 4 different sample days.

DISCUSSION

In the current study, we found a low number (< 1%) of packages with *Campylobacter* on the outer surface. This is substantially lower than the 24% reported in New Zealand in 2004 *(23)*, but close to the 3% found in the UK in 2001 *(8)*. This may be partly because in the current study we made a pointed effort to avoid leaky or wet packages. In examining just the leaky packages, we found 1 of 6 (16.7%) to have detectable *Campylobacter* on the outside of the package. From these data, we conclude that if a package of fresh broiler meat is neither leaking nor wet with exudate from other packages, it is reasonable to expect the outside surface to be free of *Campylobacter*.

Overall, the current data show that all whole carcasses had adequate exudate to sample and 93% were positive for *Campylobacter*, compared with 22% reported from frozen whole carcasses in 1984 *(6)*. Although parts had lower *Campylobacter* prevalence, many samples were positive, with or without exudate present. While the presence of exudate was not statistically linked to *Campylobacter* prevalence on parts, the exudate, when positive, could be a means of contamination in a kitchen, perhaps facilitating cross contamination as previously reported *(13, 14, 15)*. Therefore, control of exudate volume by an absorbent pad, although not predictive of the *Campylobacter* status of a packaged fresh poultry meat product, may help lessen risk in the consumer's kitchen.

Multiple sequence types of *C. jejuni* were encountered in the current study. Of the 19 *C. jejuni* STs detected, 12 have been reported to be associated with chicken, according to the Pubmlst website *(19)*, including the most common ST detected in the current work: ST 353. Ten STs of *C. coli* were detected in the current sample set, 7 of which were previously reported to be associated with chicken samples *(19)*. Interestingly, the most common *C. coli* ST recovered in the current study (ST 7818) has not been previously reported as being associated with chicken.

The current study confirms that *Campylobacter* can be found associated with packaged fresh poultry meat products, and a wide variety of *C. jejuni* and *coli* subtypes can be recovered. While not always contaminated with *Campylobacter*, packages containing exudate should be handled as a cross-contamination hazard. As long as a selected package is intact, non-leaking and dry on the outside, a consumer can feel reasonably confident that the outer surface of the package is free of *Campylobacter*.

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DISCLAIMER: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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