Genetic Diversity of *Arcobacter* and *Campylobacter* on Broiler Carcasses during Processing

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**ABSTRACT**

Broiler carcasses (*n* = 325) were sampled at three sites along the processing line (prescalding, prechilling, and postchilling) in a commercial poultry processing plant during five plant visits from August to October 2004. Pulsed-field gel electrophoresis (PFGE) was used to determine the genomic fingerprints of *Campylobacter coli* (*n* = 27), *Campylobacter jejuni* (*n* = 188), *Arcobacter butzleri* (*n* = 138), *Arcobacter cryaerophilus* 1A (*n* = 4), and *A. cryaerophilus* 1B (*n* = 31) with the restriction enzymes *Smal* and *KpnI* for *Campylobacter* and *Arcobacter*, respectively. *Campylobacter* species were subtyped by the Centers for Disease Control and Prevention PulseNet 24-h standardized protocol for *C. jejuni*. A modification of this protocol with a different restriction endonuclease (*KpnI*) and different electrophoresis running conditions produced the best separation of restriction fragment patterns for *Arcobacter* species. Both unique and common PFGE types of *Arcobacter* and *Campylobacter* strains were identified. A total of 32.8% (57 of 174) of the *Arcobacter* isolates had unique PFGE profiles, whereas only 2.3% (5 of 215) of the *Campylobacter* isolates belonged to this category. The remaining *Arcobacter* strains were distributed among 25 common PFGE types; only eight common *Campylobacter* PFGE types were observed. Cluster analysis showed no associations among the common PFGE types for either genus. Each of the eight common *Campylobacter* types consisted entirely of isolates from one sampling day, whereas more than half of the common *Arcobacter* types contained isolates from different sampling days. Our results demonstrate a far greater genetic diversity for *Arcobacter* than for *Campylobacter* and suggest that the *Campylobacter* types are specific to individual flocks of birds processed on each sampling day.

*Campylobacter jejuni* and *Campylobacter coli* are the most frequently isolated species of *Campylobacter* from cases of human bacterial enteritis worldwide (11, 25). Half of all sporadic cases of *Campylobacter* infection are thought to result from the consumption of contaminated raw or undercooked poultry and red meats (24). *Arcobacter* is closely related to *Campylobacter* and may be underestimated as a cause of foodborne enteritis in humans (21). *Arcobacter* and *Campylobacter* are frequent contaminants of broiler carcasses in poultry processing plants (2, 3, 7), and high levels of *Campylobacter* have been found on broiler chickens from the farm (31) and from retail chickens (38). *Arcobacter*, especially *Arcobacter butzleri*, is also commonly found on poultry and poultry products (6, 37). Therefore, to identify pathogen control points and design intervention strategies, a good understanding of the epidemiology of *Arcobacter* and *Campylobacter*, including contamination sources, transmission routes, and pathogen-host interactions in poultry meat products, is required.

Various typing methods have been used to investigate the genetic diversities of *Arcobacter* and *Campylobacter* species. Phenotypic methods, such as serotyping (19) and phage typing (30), have been used for identifying the source of infection. These methods are not widely available and have limitations, which include insufficient discrimination, cross-reactivity, and high levels of non-typeability (22, 23). In contrast, genetic-based methods have enhanced sensitivity and discrimination and improved availability. PCR-based methods, pulsed-field gel electrophoresis (PFGE), ribotyping, and DNA sequence-based typing of the flaA gene have been used in epidemiological studies (36). Relative to *Campylobacter*, few reports have appeared on typing methods for *Arcobacter*. To date, PFGE, PCR-based typing methods (1, 14, 20), ribotyping (17, 18), and amplified fragment length polymorphism analysis (26) have been used to characterize *Arcobacter* strains.

Fitzgerald et al. (10) reported that PFGE was superior to other typing methods tested for the discrimination of *C. jejuni* strains. The Centers for Disease Control and Prevention developed a standardized PFGE protocol for *C. jejuni* that requires a total of 24 to 30 h to perform (28). Two studies have been reported that used PFGE for *Arcobacter* by digestion with *EagI*, *SacII*, *AvaI*, and *Smal* restriction endonucleases (15, 29).

Little is known about the genetic diversity of *Arcobacter* and *Campylobacter* isolates obtained from broiler carcasses at the processing plant. The purpose of the present study was to modify PFGE methods for the characterization of *Arcobacter* and *Campylobacter* isolates at the processing plant. The purpose of the present study was to modify PFGE methods for the characterization of *Arcobacter* and *Campylobacter* isolates at the processing plant.
of A. butzleri, Arcobacter cryaerophilus 1A, and A. cryaerophilus 1B genetic diversity on broiler carcasses from a commercial poultry processing plant. The genetic diversity of the Arcobacter species was compared to that of C. coli and C. jejuni.

MATERIALS AND METHODS

Sample collection. Broiler carcasses were collected during a total of five plant visits from August to October 2004 at a commercial poultry processing plant. Carcasses were randomly chosen and collected by hand (with new latex gloves for each carcass) from three sites along the processing line: prescalding, prechilling, and postchilling. Twenty-five carcasses were collected at each site, and all carcasses sampled were from the same broiler flock during each plant visit. During the first two plant visits, only the prescald and postchill sites were sampled. Because of low isolate recovery from the postchill site, the prescald site was added for the third through fifth plant visits. This yielded a total of 125 prescald carcass samples, 75 prechill samples, and 125 postchill samples. Samples were placed in sterile plastic bags that were sealed and covered with ice in coolers for transport to the laboratory. All carcasses were subjected to a whole-carcass rinse. Feathered carcasses collected at the prescald site were rinsed by shaking with 500 ml of sterile distilled water for 60 s. Carcasses collected at prechilling and postchillling were rinsed by shaking with 100 ml of sterile distilled water for 60 s. Carcasses were then discarded. The rinses were poured into 100-ml sterile specimen cups and refrigerated at 4°C. Bacterial isolation was begun within 1 h of sample collection.

Isolation of Arcobacter. Both direct plating and enrichment methods were used for Arcobacter isolation. Serial dilutions were direct plated on cyclophosphamide, vincristine, and Adriamycin agar (5). For enrichment, 1 ml of rinse was inoculated into 5 ml of Houf broth (13). Following aerobic incubation at 25°C for 48 h, a sterile swab was used to streak a portion of the broth onto cyclophosphamide, vincristine, and Adriamycin agar. All plates from direct and enriched samples were incubated aerobically at 25°C for 48 h. Isolates were restreaked twice on brucella agar (Hardy Diagnostics, Santa Maria, Calif.) supplemented with 5% (vol/vol) lysed horse blood (Lampire Biological Laboratories, Pipersville, Pa.) to ensure clonality. Presumptive identification of Arcobacter was performed by microscopic examination of wet mounts of colonies with phase-contrast optics. Isolates were stored at −70°C in Wang freezing medium (34) with 15% (vol/vol) glycerol and brucella broth (Sigma, St. Louis, Mo.).

Isolation of Campylobacter. Isolates from prescald samples were collected for direct plating by a procedure and with a medium similar to those described for Arcobacter. For enrichment, 1 ml of rinse was placed in 5 ml of Bolton broth (4). Enrichment cultures were incubated for 24 h at 42°C in a microaerobic atmosphere consisting of 5% O₂, 10% CO₂, and 85% N₂. Following incubation, 0.1 ml of enriched Bolton broth was spread onto cyclophosphamide, vincristine, and Adriamycin agar, and these plates were incubated microaerobically for 48 h at 42°C. From each positive plate, one typical Campylobacter colony was subcultured twice on brucella agar. Isolates were presumptively identified as described for Arcobacter and stored at −70°C in Wang freezing medium (34).

Arcobacter multiplex PCR. Reference strains of Arcobacter, including A. butzleri (ATCC 49616), A. cryaerophilus 1A (ATCC 43158), A. cryaerophilus 1B (ATCC 49615), and A. skirrowii (ATCC 51132), were used as controls. Reference strains and all presumptive Arcobacter isolates were cultured on brucella agar at 25°C for 48 h under an ambient atmosphere. A modified multiplex PCR for Arcobacter (16) was used for species identification. The 50-μl PCR reaction mixture contained 25 ng of DNA template, 50 pmol of each primer set N.c. 1A and ARCO-U, 10 pmol of each primer set N.c. 1B, N. butz, and N. skir, 0.5 U of Jump Start Tag DNA polymerase (Sigma), 0.8 mM deoxynucleoside triphosphates (Applied Biosystems, Warrington, UK), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1.5 mM MgCl₂. Amplification was performed with the thermal cycler program PTC-200 (MJ Research, Watertown, Mass.) in the following order. (i) Denaturation was performed at 94°C for 10 min and was followed by 30 amplification cycles. (ii) Denaturation was performed for 30 s at 94°C, annealing for 1 min at 64°C, elongation for 1 min at 72°C, and final elongation at 72°C for 7 min. Amplification products were analyzed by electrophoresis. Gels were stained with ethidium bromide and visualized by a UV gel documentation system (Fluorochrom 8900, Alpha Innotech Corp., San Leandro, Calif.). A molecular weight standard (Roche, Indianapolis, Ind.) ranging from 100 to 1,500 bp was included on each gel.

Campylobacter BAX PCR. Reference strains for Campylobacter included C. coli (ATCC 33559) and C. jejuni (ATCC 33560). Identification of C. coli and C. jejuni was determined by the BAX PCR assay (Dupont Qualicon, Wilmington, Del.), as previously described (9). Amplification products were analyzed as described for Arcobacter.

PFGE subtyping. Ribot et al. (28) of the Centers for Disease Control and Prevention and PulseNet developed a 24-h standardized PFGE protocol for C. jejuni. The Campylobacter and Arcobacter isolates in the present study were subtyped by this protocol, although a different restriction enzyme (KpnI) and modified electrophoresis running conditions were used for Arcobacter. Salmonella Braenderup H9812 that had been restricted with XbaI was used as the molecular size standard. Salmonella Braenderup H9812 was grown on sheep’s blood agar plates (Remel, Lenexa, Kans.) at 37°C for 24 h. Frozen-stored isolates of Arcobacter and Campylobacter were streaked onto brucella agar plates. Arcobacter isolates were incubated aerobically at 25°C for 48 h; Campylobacter isolates were incubated microaerobically at 42°C for 24 to 48 h. For the restriction digestion of Arcobacter, 200 μl of a restriction enzyme mixture containing 40 U of KpnI (New England Biolabs Inc., Beverly, Mass.) was added to microfuge tubes containing prepared gel plugs and incubated at 37°C for 4 h. For Campylobacter, the sliced plugs were prerestricted in a 1× restriction buffer solution (SureCut buffer A, Roche) at 25°C for 5 min. Following prerestriction, the buffer was removed from the plug slices, and 200 μl of a restriction enzyme mixture containing 40 U of Smal (Roche) was added. The plug slices were then incubated for 4 h at 25°C.

The restriction fragments of the Arcobacter isolates were separated by PFGE (CHEF Mapper, Bio-Rad, Hercules, Calif.) on 1% agarose gels in 0.5× Tris-borate-EDTA buffer for 18 h under a constant temperature of 14°C with the AutoAlgorithm function for the following electrophoresis conditions: an initial switch time of 6.76 s, a final switch time of 13.68 s, a gradient of 6 V/cm, an angle of 120°, and a range of 30 to 400 kbp. Electrophoresis conditions for Campylobacter included a final switch time of 38.35 s and a range of 50 to 400 kbp. The gels were stained for 30 min with ethidium bromide (10 mg/μl; Sigma) and were then destained three times in sterile distilled water. Gels were visualized with a UV gel documentation system (Fluorochrom 8900, Alpha Innotech).
Analysis of PFGE patterns. The PFGE patterns of Arcobacter and Campylobacter were analyzed by the BioNumerics software program (version 3.5, Applied Maths, Austin, Tex.) and saved in tagged image file format. The optimization setting for Arcobacter and Campylobacter was 1.5%; the band position tolerance was 0.8% for Arcobacter and 0.5% for Campylobacter. Suspected double bands were checked by examining the plotted densitometric curves of the PFGE profiles. Cluster analysis was performed by the Dice coefficient and the unweighted pair group method using arithmetic averages (UPGMA). Arcobacter and Campylobacter isolates were automatically assigned to groups according to restriction pattern similarities with the routines in the BioNumerics software package. The initial groupings were manually edited by examining each PFGE pattern within a group and making appropriate changes.

RESULTS

Preliminary PFGE studies were conducted with five isolates each of Arcobacter and Campylobacter from the same broiler carcass to determine the number of genetically distinguishable isolates from a broiler carcass. For both Arcobacter and Campylobacter, a total of 25 isolates from five different broiler carcasses were analyzed, and each group of five from one carcass showed identical PFGE profiles (data not shown). On the basis of these results, one isolate per broiler carcass was selected and analyzed in this study.

The PFGE patterns of KpnI-digested genomic DNA from Arcobacter isolates (n = 174) and of SmaI-digested genomic DNA from Campylobacter isolates (n = 215) were composed of 10 to 19 and 6 to 11 fragments, respectively (Fig. 1). The genomic DNA from two Arcobacter isolates (one A. butzleri and one A. cryaerophilus 1B) was not cut by KpnI. These isolates were thus nontypeable, but all Campylobacter isolates yielded usable patterns.

The Arcobacter isolates were distributed among a total of 82 unique and common PFGE types. Unique PFGE types made up 32.8% (57 of 174) of all Arcobacter strains. Among Arcobacter species, A. cryaerophilus 1B showed the highest proportion of isolates that could be placed in the unique category (61.3% [19 of 31]), followed by A. cryaerophilus 1A (50.0% [2 of 4]) and A. butzleri (25.9% [36 of 139]). Of the 25 common PFGE types, one was A. cryaerophilus 1A, and four were A. cryaerophilus 1B (Table 1). The majority (20 of 25) of the common Arcobacter types were composed of A. butzleri and were by far the most commonly isolated Arcobacter species in this study. Isolates of the common A. butzleri types made up 74.1% (103 of 139) of all the A. butzleri strains isolated. Cluster analysis demonstrated that the Arcobacter common PFGE types were highly diverse (Fig. 2). No major clusters were observed, and the isolates did not cluster by species, sampling day, or collection site. Also, 52% (13 of 25) of the groups contained isolates collected on different sampling days (Fig. 2).

In contrast to the diversity of the Arcobacter strains, the Campylobacter strains were divided among just 13 unique and common PFGE types (Table 2). Only 2.3% (5 of 215) of the Campylobacter isolates belonged to unique PFGE types. The percentage of C. coli strains with unique PFGE types was slightly higher than that of C. jejuni strains (Table 2). The eight common PFGE types included one C. coli, which made up 96.3% (26 of 27) of the C. coli isolates in this study. The other seven common PFGE types of Campylobacter made up 97.9% (184 of 188) of the C. jejuni isolates. Cluster analysis showed that the Campylobacter common PFGE types were also quite diverse, although much less so than those of Arcobacter (Fig. 3). As with Arcobacter, no major clustering was observed, nor did the isolates cluster by species, sampling day, or collection site. However, in contrast to Arcobacter, each Campylobacter PFGE type was composed of isolates collected on a single sampling day (Fig. 3).

<table>
<thead>
<tr>
<th>Species</th>
<th>Unique types</th>
<th>Common types</th>
</tr>
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<tbody>
<tr>
<td>A. butzleri</td>
<td>36 (25.9%)</td>
<td>20 (74.1%)</td>
</tr>
<tr>
<td>A. cryaerophilus 1A</td>
<td>2 (50.0%)</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>A. cryaerophilus 1B</td>
<td>19 (61.3%)</td>
<td>4 (12.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>57 (32.8%)</td>
<td>25 (67.2%)</td>
</tr>
</tbody>
</table>

1 Number of types in each category.
2 For A. butzleri, n = 139; A. cryaerophilus 1A, n = 4; A. cryaerophilus 1B, n = 31; total isolates, n = 174.
DISCUSSION

PFGE was used to characterize 174 arcobacters and 215 campylobacters isolated from 325 broiler carcasses in a commercial poultry processing plant. Digestion of the Arcobacter genomic DNA with KpnI yielded PFGE patterns that showed a high discrimination, which was useful for differentiating the genotypes. A. butzleri, A. cryaerophilus 1A, and A. cryaerophilus 1B isolates from the three different collection sites in the processing facility were distinctly different from each other. The Arcobacter isolates showed a much higher percentage of unique PFGE types (32.8% [57 of 174] versus 2.3% [5 of 215]) than the Campylobacter isolates. More than half of the A. cryaerophilus 1B strains were found in unique PFGE types (61.3% [19 of 31]). Furthermore, a larger number of common PFGE types were found for Arcobacter (25 groups) than for Campylobacter (eight groups). These results indicate that the carcasses from this poultry processing plant were colonized by a number of different strains of Arcobacter. Because Arcobacter is aerobic and grows at 25°C, this could be the result of in-plant contamination by strains acquired by the carcasses as they proceeded along the processing line. However, the diversity of isolates from the prechill and postchill sites was not greater than from the prescald site. A more likely source of the Arcobacter strain diversity is the broiler grow-out facility where the birds originated. These facilities probably harbor a number of different Arcobacter genotypes that contaminate the birds. In contrast, only one or a few Campylobacter strains appear to become dominant within a particular broiler flock, as evidenced by the limited diversity observed with respect to the day of sample collection.

Hume et al. (15) reported that there was little similarity between genotypic patterns produced by AvaI-, EagI-, and SacII-digested DNA from Arcobacter isolates collected from a farrow-to-finish swine facility. Of these three restriction enzymes, EagI and SacII produced PFGE patterns better suited for differentiation than AvaI. Hume et al. concluded that the observed genotypic variation suggested that the pigs sampled in their study were colonized by multiple

**TABLE 2. Distributions of PFGE profiles of Campylobacter on broiler carcasses from the poultry processing plant**

<table>
<thead>
<tr>
<th>Species</th>
<th>Unique types</th>
<th>Common types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Types</td>
<td>Isolates (%)</td>
</tr>
<tr>
<td>C. coli</td>
<td>1</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>4</td>
<td>4 (2.1)</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>5 (2.3)</td>
</tr>
</tbody>
</table>

a Number of types in each category.
b For C. coli, n = 27; C. jejuni, n = 188; total isolates, n = 215.
Arcobacter genotypes, perhaps resulting from genomic rearrangements among parent strains. Rivas et al. (29) characterized A. buzleri isolates from ground poultry, pork, beef, and lamb retail meats by PFGE through the independent use of the restriction enzymes SacII, EagI, and SmaI to cut each isolate. They determined that common Arcobacter types epidemiologically related a number of isolates with indistinguishable PFGE patterns, suggesting cross-contamination of the meat samples. However, the enzymes used for PFGE generated only four to eight DNA fragments per isolate. In preliminary PFGE studies for the present report, Avai, EagI, and SacII enzymes were tested, but the typeability and discrimination among Arcobacter isolates was low (data not shown). With KpnI, 10 to 19 fragments per strain were produced, which enabled better genotypic discrimination among Arcobacter isolates in the present study than observed in these previous reports (15, 29).

PFGE results can be influenced by the technician analyzing the band patterns, by the software program(s) used, and by the criteria used to differentiate epidemiologically related and unrelated isolates (33). In the present study, alternative typing methods were investigated before PFGE was chosen (data not shown). Enterobacterial repetitive intergenic consensus PCR and random amplified polymorphic DNA PCR have been used for the genetic typing of Arcobacter isolated from poultry products or mechanically separated turkey (14, 20). However, when comparing PFGE, enterobacterial repetitive intergenic consensus PCR, and random amplified polymorphic DNA PCR were used for the genetic typing of Arcobacter isolated from retail chicken. However, multiple genotypes of C. jejuni have been isolated from a commercial broiler flock during rearing and even from the gastrointestinal tracts of individual birds (12). Furthermore, a Danish study (27) showed that C. jejuni can persist during successive broiler flock rotations. In our study, the one C. coli and seven C. jejuni common PFGE types were differentiated with respect to sampling days but not sampling site.

The number of unique PFGE types of Arcobacter varied by sampling site. The percentages of unique PFGE types in Arcobacter strains at the three sites along the processing line were (i) prescalding, 22% (26 of 116); (ii) prechilling, 52% (24 of 46); and (iii) postchilling, 58% (7 of 12). Only five unique PFGE types were observed for Campylobacter. In contrast to Campylobacter, processing appeared to have an effect on the diversity of Arcobacter. In our study, Arcobacter strains from postchilling showed greater genetic diversity than Campylobacter strains from the same processing site.

Although PFGE is somewhat labor-intensive, we found it very useful for comparing the genotypic diversity of Arcobacter and Campylobacter. PFGE relies on polymorphisms occurring throughout the genome and has a higher discriminatory power than other genetic typing methods (10). For global epidemiological studies, the standardization of procedures is necessary. A Campylobacter genotypic database is currently being compiled by PulseNet, which will allow rapid analyses and comparisons of PFGE patterns from different sources (32). Likewise, a genotypic database of Arcobacter by PFGE with KpnI as the restriction enzyme would help provide more accurate information with respect to the sources of isolates. Future studies that involve genotyping Arcobacter and Campylobacter strains from several flocks on the same sampling day have been proposed to address whether cross-contamination occurs between flocks during processing.

In conclusion, PFGE for Arcobacter can assist in tracking Arcobacter suspected in foodborne disease outbreaks. PFGE profiling is a valuable tool for the taxonomic and epidemiological analysis of Arcobacter and Campylobacter strains.
ACKNOWLEDGMENTS

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REFERENCES


