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Prevalence and Genetic Variability of *Arcobacter* Species in Mechanically Separated Turkey†‡

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ABSTRACT

A survey for *Arcobacter* spp. and *Arcobacter butzleri* in mechanically separated turkey was conducted during the winter of 1995 and summer and fall of 1996. *Arcobacter* spp. and *A. butzleri* were identified by polymerase chain reaction and species-specific oligonucleotide probes. *Arcobacter* spp. were isolated from 77% (303 out of 395) of the mechanically separated turkey samples with 74% (223 out of 303) of these samples positive for *A. butzleri*. Of the 121 *A. butzleri* isolates tested, 86 different patterns were evident following amplification of enterobacterial repetitive intergenic consensus sequences. The extent of genetic polymorphism indicated multiple sources of contamination.

Aerotolerant, vibrio-like organisms were first isolated from aborted bovine and porcine fetuses (8, 9, 22) and classified as *Campylobacter cryaerophilus* (21). After morphological (21), biochemical (21), and phenotypic (14) characterizations, as well as DNA–DNA (14) and DNA–rRNA hybridizations (28), it was proposed that *C. cryaerophilus* be placed into the new genus, *Arcobacter* (28). The four species of *Arcobacter* include *A. cryaerophilus* (subgroups 1A and 1B), *A. butzleri*, *A. skirrowii*, and *A. nitrofigilis* (31).

The epidemiology of *Arcobacter* spp. is not fully understood. *A. cryaerophilus* organisms have been isolated from aborted as well as healthy livestock (23) and from human stool samples (14, 26). *A. butzleri* has been cultured from animals (1, 19, 22, 32–34) and from humans with diarrhea and/or abdominal cramps (14, 15, 18, 26, 27, 30). The clinical symptoms of *A. butzleri* suggest that it is a human pathogen (14, 15). *A. butzleri* organisms have also been isolated from water (6, 10, 13), poultry (2, 5, 10, 16, 20), and pork (4, 5).

Various enrichment methods have been used to recover *Arcobacter* spp. from meats (2, 4, 5, 16). The *Leptospira* semisolid medium, Ellinghausen–McCullough–Johnson–Harris Polysorbate-80 (EMJH P-80), was first used to detect *Arcobacter* in aborted livestock fetuses (8, 9). EMJH P-80 has also been used to enrich for *Arcobacter* spp. in ground pork (4). In that study, *Arcobacter* spp. was isolated from 89.9% of the pork samples (n = 149) tested during the first survey and 90.0% of the pork samples (n = 30) tested during the second survey (4) from a single pork-processing establishment. In contrast, only 5% of the samples (n = 120) obtained from four other pork-processing establishments yielded *Arcobacter* spp.

The *Arcobacter* Selective Broth and the *Arcobacter* Selective Medium developed by de Boer et al. have been used to enrich for *Arcobacter* spp. in poultry, beef, and pork (5). Using this protocol, *Arcobacter* spp. were isolated from 24.1% of the poultry samples (n = 220) tested with lower recoveries, 4.9% and 0.5% for beef and pork, respectively (5). Lammerding used a modified Rosef broth to enrich for *Arcobacter* spp. in poultry products (16). Using this method, *A. butzleri* was isolated from 96.8% of the broiler chicken carcasses (n = 125) and from 85.7% of the fresh ground turkey samples (n = 7) (16).

Proper identification of *Arcobacter* is needed in order to understand fully its role in causing human foodborne illness. The morphological similarities between *Arcobacter* spp. and *Campylobacter* spp. may lead to the misidentification of the organisms when relying on the traditional plating methods and dark-field microscopy. The two organisms do show some physiological differences: *Arcobacter* spp. grow at 15°C in the presence of oxygen and in 1.5% NaCl, whereas *Campylobacter* spp. require growth at 37°C under microaerobic conditions (3 to 10% oxygen) (28).

The use of oligonucleotide DNA probes (32) and polymerase chain reaction (PCR)-based methods (3, 12) provides an alternative method to identify *Arcobacter* spp. These methods are based on identifying sequences that are specific for the 16S rRNA or 23S rRNA genes of *Arcobacter* spp. and *A. butzleri* (3, 12, 34). PCR-based DNA fingerprinting has elucidated the epidemiology of *Arcobacter* (29).

This method relies on amplification of the enterobacterial repetitive intergenic consensus (ERIC) sequences found in gram-negative organisms. The resultant DNA patterns allow for the differentiation of the isolates (31). In one study, DNA
fingerprints of outbreak-related strains of *A. butzleri* were identical (29). In contrast, multiple patterns would indicate strain differences and thus suggest more than one source of contamination. Despite the application of PCR-based ERIC fingerprinting in epidemiological studies, the function of these repetitive DNA sequences is unknown.

The Nationwide Raw Ground Turkey Microbiological Survey conducted by the Food Safety and Inspection Service (FSIS) found that 25.4% of the raw ground turkey samples (*n* = 295) were contaminated with *Campylobacter jejuni/coli* (11). Other surveys on turkey products indicate that *C. jejuni* contamination ranges from 0 to 90% (17, 35). Since *Arcobacter* is closely related phylogenetically to and is more aerotolerant than *Campylobacter*, it is plausible that *Arcobacter* may also be found at high levels in poultry. In two different studies, *Arcobacter* was isolated from 24.1% of poultry samples (*n* = 220) (5) and six of seven fresh ground turkey samples (16). In a pilot study on turkey skin samples conducted in our laboratory, all samples (*n* = 12) were found to be positive for *A. butzleri* (4).

The morphological similarities between *Arcobacter* spp. and *Campylobacter* spp. as well as their presence in turkey products led to surveying mechanically separated turkey. Mechanically separated turkey is widely used in the production of both cooked and raw meat products. The presence of *Arcobacter* spp. in mechanically separated turkey could represent a potential foodborne hazard.

The objective of this study was to determine the prevalence of *Arcobacter* spp. and *A. butzleri* in mechanically separated turkey. In addition, the summer and fall *A. butzleri* isolates were analyzed for genetic variation in ERIC sequences.

**MATERIALS AND METHODS**

**Sampling techniques.** Three surveys were conducted. The winter (initial) survey consisted of 100 mechanically separated turkey samples that were obtained from a poultry plant (A) on four separate dates in January and February 1996. Two additional surveys, summer and fall 1996, were conducted after the initial survey data were analyzed. The summer and fall surveys were expanded to include the initial plant (A) along with two additional plants (B and C) in two different states. The summer survey consisted of 145 mechanically separated turkey samples. 25 samples from each plant (except plant B, which furnished 45 samples), collected on two separate dates in July and August 1996. The fall survey consisted of 150 mechanically separated turkey samples. 25 samples from each plant collected on two separate dates in September 1996. The mechanically separated turkey used in the surveys was typical of that used in the meat industry. It consisted of a fresh homogenous mixture composed of skeletal tissue, skin, and nonmeat ingredients (salt and sodium nitrite). All samples were collected using the same method: 10 g of each mechanically separated turkey sample were enriched in 50-ml plastic centrifuge tubes (Blue Max, Becton Dickinson, Lincoln Park, N.J.) containing 20 ml of EMJH P-80 semisolid media (7) supplemented with agar and 100 mg of 5-fluorouracil per liter (4, 22).

**Arcobacter spp. enrichment techniques.** Figure 1 shows a general schematic flow diagram for the identification of *Arcobacter* spp. and *A. butzleri* for the winter mechanically separated turkey samples.

**FIGURE 1. General schematic flow diagram for the identification of Arcobacter spp. and A. butzleri for the winter mechanically separated turkey samples.**

**FIGURE 2. General schematic flow diagram for the identification of Arcobacter spp. and A. butzleri for the summer and fall mechanically separated turkey samples.**

**Mechanically Separated Turkey Samples**

- Enrichment in P-80 (7 Days, 30° C)
- Subcultured in P-80 (3 Days, 30° C)
- PCR to Identify Arcobacter spp. Positive Samples
- Hybridization to Identify A. butzleri Positive Samples
- DNA Fingerprinting of A. butzleri Positive Samples

- Southern Transfer of PCR Gel

- PCR to Identify Arcobacter spp. Positive Samples
- Extraction of DNA from the Arcobacter spp. Positive Samples
- DNA Dot Blot Hybridization with A. butzleri-specific Probe
- DNA Fingerprinting of A. butzleri Positive Samples

Sampling techniques. Three surveys were conducted. The winter (initial) survey consisted of 100 mechanically separated turkey samples that were obtained from a poultry plant (A) on four separate dates in January and February 1996. Two additional surveys, summer and fall 1996, were conducted after the initial survey data were analyzed. The summer and fall surveys were expanded to include the initial plant (A) along with two additional plants (B and C) in two different states. The summer survey consisted of 145 mechanically separated turkey samples, 25 samples from each plant (except plant B, which furnished 45 samples), collected on two separate dates in July and August 1996. The fall survey consisted of 150 mechanically separated turkey samples, 25 samples from each plant collected on two separate dates in September 1996. The mechanically separated turkey used in the surveys was typical of that used in the meat industry. It consisted of a fresh homogeneous mixture composed of skeletal tissue, skin, and nonmeat ingredients (salt and sodium nitrite). All samples were collected using the same method: 25 samples were sent per date with five samples (75 g each) from each of five randomly selected containers of product ("combos", ~900 kg each). The samples were collected by plant personnel and were shipped overnight on ice to the National Animal Disease Center, Ames, Iowa. Flow diagrams for the isolation and identification of *Arcobacter* spp. and *A. butzleri* for the winter (Fig. 1) and for the summer and fall surveys (Fig. 2) are given.
The winter samples were enriched (7 days, 30°C), subcultured (1 ml of enrichment into 9 ml of fresh EMJH P-80), and incubated for an additional 3 days at 30°C. The summer and fall samples were enriched (3 days, 30°C), subcultured (1 ml of enrichment into 9 ml of fresh EMJH P-80), and incubated (3 days at 30°C).

Pure cultures were obtained as follows: A 0.5-ml aliquot from the EMJH-P-80 enrichment was filtered onto 0.45-μm membranes placed onto the surface of brain–heart infusion agar (Difco) supplemented with 10% defibrinated bovine blood. After 30 min, the membranes were removed, the filtrate was streaked for colony isolation, and plates were incubated aerobically (30°C for 48 to 72 h).

**Arcobacter spp. identification.** For all surveys, a 250-μl aliquot (previously frozen) of each subculture was used to perform the PCR reaction for the detection of *Arcobacter* spp. The aliquots were boiled (15 min, 110°C) and centrifuged (1 min, 11,000 × g). A 5-μl aliquot of the supernatant served as the PCR template. The reagents and conditions for the PCR reaction were as described (12). The amplified DNA product was analyzed by gel electrophoresis (120 V, 1 h) on a 1.5% agarose gel (Seakem ME agarose, FMC Bioproducts, Rockland, Maine) using a 6.5 × 10-cm horizontal gel bed (Minnie the Gel-Cicle, Hoefer Scientific Instruments, San Francisco, Calif.) and TBE (0.09 M Tris, 0.09 M boric acid, 0.002 M EDTA, pH 8.5) as the running buffer. The gel was stained with ethidium bromide, visualized with UV light, and photographed as described previously (12).

**Arcobacter butzleri identification.** For the winter survey, the agarose gel containing the *Arcobacter* spp. amplicons was denatured in 0.5 M NaOH, 1.5 M NaCl (30 min, 4°C), neutralized in 1 M Tris-base, 1.5 M NaCl, pH 5.5 (30 min, 4°C), and transferred onto a nylon membrane (Nytron, Schleicher & Schuell, Keene, N.H.) using the method of Southern (25) with the Turboblotter Rapid Downward Transfer System (Schleicher & Schuell). After transfer, the membrane was placed on filters saturated with 0.4 N NaOH (1 min) and then on filters saturated with 0.025 M NaOHPO₄ (1 min). The immobilized DNA on the membrane was crosslinked on both sides using the UV Stratalinker 1800 (Stratagene, La Jolla, Calif.).

The membrane was prehybridized (3 h, 37°C) using the Genius System hybridization solution (Boehringer Mannheim, Indianapolis, Ind.) and then hybridized (18 h, 37°C) with the Genius System hybridization solution containing the digoxigenin (DIG)-labeled *A. butzleri* species-specific probe (34). After hybridization, the membranes were washed and incubated with the DIG detection system according to the manufacturer’s directions. The membrane was exposed (60 min, room temperature) to X-ray film (X-Omat, Kodak, Rochester, N.Y.) and developed using the X-Omat Film Processor (Kodak).

Isolates of *Arcobacter* spp. from the summer and fall surveys were identified as *A. butzleri* by dot-blot hybridization using cesium–chloride-purified DNA and the species-specific probe (34). Purified DNA (2 μg) was immobilized on nylon membranes (Nytron, Schleicher & Schuell) as described (34). *A. butzleri* served as a positive control, whereas *A. cryaerophilus* 1A and 1B served as negative controls for the assay. The membrane was probed with the *A. butzleri*-specific oligonucleotide probe, washed, and exposed to X-ray film as described above.

**DNA fingerprinting of isolates.** Genetic variability of the field strains was determined by PCR with primers targeting the ERIC motifs. The DNA from 121 of the summer and fall *A. butzleri* isolates was amplified using primers, ERIC 1R (5′-ATG-T AA-GCT-CC T-GG-GAT-TCA-C-3′) and ERIC 2 (5′-AAG-TAA-GTG-CTT-GGG-ATC-3′) as described (29). The 50-μl PCR reaction mixture consisted of 25 pmol each of ERIC 1R and ERIC 2, 10 mmol liter⁻¹ Tris–HCl, 50 mmol liter⁻¹ KCl, 2.0 mmol liter⁻¹ MgCl₂, and 200 mmol liter⁻¹ each of the four dNTPs and 1.25 U of Taq polymerase (Boehringer Mannheim). PCR was performed in a thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) using previously described conditions (12). The PCR product was analyzed by gel electrophoresis as described above. The photographs of the gels were scanned using the Gel Doc 1000 (Bio-Rad, Hercules, Calif.) and profiles analyzed using the Molecular Analyst Software (Bio-Rad). This allowed for molecular weight values to be assigned to each of the isolates for comparison of banding patterns. Based on the molecular weights and visual inspection of the photographs, differences between isolates were obtained.

**Statistical analysis.** To determine if the incidence of *Arcobacter* and *A. butzleri* was significantly different in samples obtained from the three plants, a chi-square test of independence was conducted. *P* values of 0.05 or less were considered statistically significant. To determine if the incidence of *A. butzleri* was disproportionately distributed between the three plants, a binomial test for distribution was conducted. A binomial test *P* value of 0.05 was considered statistically significant (24).

**RESULTS**

The initial (winter) survey consisted of samples from only plant A. *Arcobacter* spp. were isolated from 92% (92 of 100) of the samples with a total of 87% (80 of 92) positive for *A. butzleri*. A representative dot-blot hybridization, which was used to identify *A. butzleri*, is shown in Figure 3. The high contamination rate of *Arcobacter* in meat samples obtained from plant A in the winter survey led to summer and fall surveys in which the same plant plus two additional plants (B and C) were tested. Based on the three samplings, plant A had 96% (191 of 200) of the samples positive for *Arcobacter* spp.; 80% (153 of 191) of these were identified as *A. butzleri* (Table 1). For plant B, 72% (68 of 95) of the samples were positive for *Arcobacter* spp. with 65% (44 of 68) identified as *A. butzleri* (Table 1). For plant C 44% (44 out of 100) of the samples were positive for *Arcobacter* spp. with 59% (26 of 44) positive for *A. butzleri* (Table 1). A total of 77% (303 of 395) of the samples were positive for *Arcobacter* spp. with 74% (223 of 303) of these confirmed as *A. butzleri* (Table 1).

![FIGURE 3. Dot-blot hybridization of Arcobacter butzleri DNA of field strains isolated from mechanically separated turkey and hybridized with the A. butzleri species-specific probe. Each sample was analyzed in duplicate. Wells A1–4 contain Arcobacter cryaerophilus (negative controls). Wells A5 to 6 contain A. butzleri (positive control). Wells B1 to D12 contain field isolates. Wells A7 to 12 are empty.](image-url)
TABLE 1. Recovery of Arcobacter spp. and A. butzleri isolated from mechanically separated turkey

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. positive Arcobacter/ no. samples tested (%)</th>
<th>No. positive A. butzleri/ no. A. butzleri samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>191/200 (96)</td>
<td>153/191 (80)</td>
</tr>
<tr>
<td>B</td>
<td>68/95 (72)</td>
<td>44/68 (65)</td>
</tr>
<tr>
<td>C</td>
<td>44/100 (44)</td>
<td>26/44 (59)</td>
</tr>
<tr>
<td>Total</td>
<td>303/395 (77)</td>
<td>223/303 (74)</td>
</tr>
</tbody>
</table>

a Tested in winter, summer, and fall.
b Tested in the summer and fall.
c Combined data from all three plants.

The recovery of Arcobacter spp. using a 7-day (winter samples) versus 3-day (summer and fall) primary enrichment was compared using the chi-square test for independence. For plant A, the only facility sampled in the winter, 92% (92/100) of samples yielded Arcobacter; 80% (80/100) yielded A. butzleri. In the summer and fall surveys, for plant A, Arcobacter spp. were detected in 99% (99/100) of the samples collected; A. butzleri was detected in 73% (73/100) of the samples. Thus, the shorter primary enrichment used for the summer and fall samples yielded a statistically significant increase in the recovery of Arcobacter spp. (P < 0.04). The shorter primary enrichment, however, yielded no statistically significant differences in the recovery of A. butzleri (P > 0.20).

The isolation rates of Arcobacter and A. butzleri for all three plants were compared using the chi-square test for independence. Differences in recovery of Arcobacter from plants A, B, and C were statistically significant (P < 0.01). Inspection of the data suggested that A. butzleri was recovered at higher levels in some of the plants. The binomial distribution test for recovery of A. butzleri was compared for all three facilities. Except for plant C (59.1%; P > 0.25), there was a disproportionately higher number of samples contaminated with A. butzleri from plants A (64.7%; P < 0.01) and B (80.1%; P < 0.05).

A total of 121 summer and fall A. butzleri isolates were analyzed for distinct DNA amplification patterns by PCR-based DNA fingerprinting (29). Eighty-six different patterns were obtained from the 121 isolates. A representative set of DNA profiles is shown in Figure 4. Twenty of the 86 patterns were repeated at least twice in either the same plant or in two different plants. Overall, 71% (86 different profiles for 121 isolates) of the isolates displayed unique DNA amplification patterns. In plant A, 64% (38 different patterns for 59 isolates) of the isolates displayed unique patterns. In plant B, 89% (32 different patterns for 36 isolates) of the isolates displayed unique patterns. In plant C, 81% (21 different patterns for 26 isolates) of the isolates displayed unique patterns.

DISCUSSION

Arcobacter spp. (77%) and A. butzleri (56%) were present in a total of 395 samples of mechanically separated turkey obtained from three processing sites. Species were confirmed by dot-blot hybridization with the A. butzleri species-specific probe (34). Differences in the recovery rate between plants were noted. Plant A had the highest recovery for Arcobacter spp. (96%) of which 80% of the positive samples were confirmed as A. butzleri. Plant C yielded the lowest percentage of Arcobacter (44%) with 59% (26 of 44) of these positive for A. butzleri. Differences in recovery of Arcobacter were reported earlier for ground pork obtained from various sources (4). Collins et al. detected Arcobacter in 89% of ground pork samples (n = 149) obtained from a slaughter facility (plant 1). In a later survey involving plant 1 and four other premises (plants 2 through 5), 90% of samples from plant 1 were again positive, but only 5% of the total 120 samples from the four other facilities (plants 2 through 5) yielded Arcobacter spp. (4).

Significant differences were found in the recovery of Arcobacter between plants A, B, and C. In addition, a disproportionately high number of A. butzleri strains were recovered in plants A and B but not in C. Sources of turkeys and differences in plants (geographic location, age) could all account for the variations. In an earlier study, it was not determined whether management practices at the source farms or the sanitary conditions during hog slaughter influenced the prevalence of Arcobacter spp. in ground pork (4). Likewise, in this study, although the causes of contamination of the turkey product were not explored, variations between plants could be due to several factors, including the source of the birds, slaughter practices, and environmental contamination during processing. Thus, the factors contributing to these differences are unknown.

Although few surveys have been conducted for the presence of aerotolerant Campylobacter-like organisms in meats, Arcobacter, like Campylobacter has been reported from poultry (2, 5, 10, 16, 17, 20). In France, A. butzleri was recovered from 81% of poultry carcasses examined (n = 201). Nearly half of the poultry isolates in that study were of serogroup 1 (20). In a survey of poultry products in

FIGURE 4. Representative ERIC-based DNA fingerprints of Arcobacter butzleri. Lane 1, molecular weight marker VI (Boehringer Mannheim) was used for size comparison (bp). Lanes 2 to 7 and 9 to 12 contain A. butzleri field strains. Lane 8 is blank. Lane 13 is a PCR negative control.
Canada, *A. butzleri* was recovered from 97% (121 of 125) of poultry carcasses obtained from five different processing plants. In addition, *A. butzleri* was cultured from retail-purchased whole and ground poultry, chicken, and turkey samples (16). As was the case in the French study, serotype I was the predominant serotype isolated from Canadian poultry (16). In contrast, *Arcobacter* was detected in only 24% (53 of 224) of retail-purchased poultry in the Netherlands (5).

Differences in published recovery rates could be attributed to multiple factors, such as bias in plant selection, hygienic conditions throughout production and processing, or differences in the sensitivity of isolation methods. In the current study, to expedite the identification of *Arcobacter* spp., the length of primary enrichment was reduced from 7 days (winter survey) to 3 days (summer and fall surveys). The shorter primary enrichment used for the summer and fall samples yielded a statistically significant increase in the recovery of *Arcobacter* spp. (*P* < 0.04). The shorter primary enrichment, however, yielded no statistically significant differences in the recovery of *A. butzleri* (*P* > 0.20).

In earlier studies, PCR-mediated DNA fingerprinting confirmed the genetic identity of *A. butzleri* isolates recovered from a nursery school outbreak and suggested a single source of contamination (29). In this study, PCR-based fingerprinting was used to distinguish the field strains of *A. butzleri*. By PCR amplification of repetitive ERIC sequences, 71% of the DNA profiles of the summer and fall isolates of *A. butzleri* were unique. The multiple DNA fingerprints may indicate numerous sources of contamination. Interestingly, while plant A had the highest recovery for both *Arcobacter* spp. (96%) and *A. butzleri* (76%), it exhibited the lowest percentage (64%) of polymorphism in the ERIC sequences. Thus, plant A had more isolates with similar DNA profiles than the other plants examined. Further, plant C, with only 26 *A. butzleri* isolates, exhibited a disproportionately high number (*n* = 21) of distinctive profiles.

In conclusion, this study shows that *Arcobacter* spp., especially *A. butzleri* is prevalent in mechanically separated turkey. The diversity of DNA patterns found among the *A. butzleri* isolates suggests multiple sources of contamination. Thus, future studies should focus on the source of contamination, seasonal and geographical variations, and plant sanitation practices to reduce contamination.

**ACKNOWLEDGMENTS**

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