Prevalence and diversity of *Arcobacter* spp. in retail chicken meat in Turkey

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Abstract

Arcobacters are food and waterborne pathogens associated with human and animal infections. The objective of the present study was to investigate the prevalence and diversity of *Arcobacter* spp. in commercially sold chicken meat in Izmir region of Turkey. For this purpose, 100 samples including legs (n=40), 17 chicken quarters (n=17), drumstickers (n=16), breasts (n=11), wings (n=10), and carcasses (n=6) were collected from different retail markets. A total of 65 isolates were confirmed as *Arcobacter* spp. from 55 samples by genus-specific polymerase chain reaction (PCR). The prevalence of *Arcobacter* spp. was 32.5, 81.3, 64.7, 72.7, 83.3, and 50% for legs, drumstickers, chicken quarters, breasts, carcasses and wings, respectively. Based on the multiplex-PCR, most of the isolates were identified as *A. butzleri* (n=45, 80%), followed by *A. cryaerophilus* (n=2, 3.6%), *A. skirrowii* (n=1, 1.8%) and 17 isolates (30.9%) could not be identified at the species level.

Materials and Methods

Bacterial strains

The bacterial reference strains *A. butzleri* (LMG 10828), *A. cryaerophilus* 1A (LMG 9904) and 1B (LMG 10229), and *A. skirrowii* (LMG 6621) were kindly provided by Maria José Figueras (University of Rovira i Virgili, Spain).

Sample collection

Samples used in this study [legs (n=40), chicken quarters (n=17), drumstickers (n=16), breasts (n=11), wings (n=10), and carcasses (n=6)] were collected from a number of retail supermarkeths in Izmir, Turkey. All samples were transported to the laboratory immediately, kept cool and analyzed within 24 h.

Isolation

Each sample (10 g) was homogenized for 1-2 min in 90 mL of sterile buffered peptone (Oxoid CM0509, UK). Then, an aliquot of the homogenate (10 mL) was inoculated into equal volume of double strength *Arcobacter* broth (Oxoid CM965, UK) containing cefoperazone-amphotericin B-teicoplanin selective supplement (CAT, Oxoid SR174E, UK). This suspension was incubated microaerobically for 48 h (Anoxomat Mart II Microbiology, The Netherlands) at 30°C. After enrichment, the sample (100 µL) was filtered using 0.45-µm pore size nitrocellulose membrane filters (Millipore HAWG047S1, USA) onto the modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid CM739, UK). After incubation at 30°C for 45 min, the filtrate was streaked onto mCCDA plates. Finally, the plates were incubated at 30°C aerobically for 48 h.12 Suspected colonies from each plate were purified by streaking and confirmed by Gram-staining, oxidase tests (Merck 1.13306, Germany), and motility under phase contrast microscope. The isolates that were Gram-negative, spiral-shaped, motile and oxidative-positive isolates were stored in 20% (*v/v*) nutrient broth No.2 (Oxoid CM0067, UK)-glycerol (AppliChem A1123, Germany) at -80°C.

Genus-specific polymerase chain reaction

DNA was extracted using a commercial genomic DNA isolation kit (PureLink® Kit, Invitrogen, Thermo Fisher Scientific, USA). The primers of Harmon and Wesley (1996)23 were used for genus-specific polymerase chain reaction (PCR). The reaction was performed in a total volume of 25 µL containing 2 µL template DNA, 2.5 µL of 10×PCR buffer (750 mM Tris-HCl (pH 8.8), 200 mM MgCl₂, 0.1% (*v/v*) Tween 20, and 1.5 mM dNTP) of each of the primers, 0.2 mM each of the four dNTPs (Fermentas, Thermo Fisher Scientific, USA) and 1.5 U Taq DNA polymerase (Fermentas, Thermo Fisher Scientific, USA). The samples were subjected to an initial denaturation step (94°C for 5 min), followed by 35 amplification cycles. Each amplification cycle consisted of 1 min at 94°C (denaturation), 1 min at 56°C (primer annealing), and 1 min at 72°C (primer extension). A primer extension step (72°C for 7 min) followed the final amplification cycle. PCR experiments were repeated twice for each strain. The amplified products were resolved in 1% (*w/v*) Tris-acetate-EDTA (TAE) agarose gel and the band patterns were analyzed in the gel...
documentation system (Vilber Lourmat, France). DNA from reference strains were used as positive controls and sterile distilled water served as negative control.

**Multiplex-PCR**

The isolates identified as *Arcobacter* spp. were examined by m-PCR using the primers developed by Houf and colleagues.²⁴ PCR reactions were performed in a 25 µL reaction mixture containing 2 µL template DNA, 2.5 µL of 10×PCR buffer (750 mMol/L Tris-HCl (pH 8.8), 200 mMol/L (NH₄)₂SO₄, 0.1% (v/v) Tween 20, and 1.5 mMol/L MgCl₂), 10 mMol/L of each of the primers, 0.2 mMol/L each of the four dNTPs (Fermentas, Thermo Fisher Scientific, USA) and 1.0 U Taq DNA polymerase (Fermentas, Thermo Fisher Scientific, USA). PCR involved initial denaturation at 94°C for 3 min, 35 cycles of denaturation (94°C, 45 s), primer annealing (60°C, 45 s) and chain extension (72°C, 1 min), and final extension (72°C, 7 min). The amplified products were resolved in 1% (w/v) TAE agarose gel and the band patterns were analyzed in the gel documentation system (Vilber Lourmat, France). DNA from reference strains were used as positive controls and sterile distilled water was used as negative control.

**Results and Discussion**

Since it is difficult to identify *Arcobacter* species using cultural methods, molecular detection and identification methods have been developed for *Arcobacter* spp.²⁵ In the present study, we determined the prevalence and diversity of *Arcobacter* spp. in chicken samples collected from Izmir region of Turkey by PCR-based methods.

Based on the genus-specific PCR, 65 isolates were identified as *Arcobacter* spp. among 100 samples analyzed (Table 1). In total, 55 samples were positive for the presence of *Arcobacter* spp. (55%). The highest occurrence was obtained in carcasses (n=5, 83.3%), followed by drumstickers (n=13, 81.3%), breasts (n=8, 72.7%), leg quarters (n=11, 64.7%), wings (n=5, 50%) and legs (n=13, 32.5%). Studies indicated that the prevalence of *Arcobacter* spp. in poultry ranged from 38.4 to 90.6% and the overall prevalence (55%) obtained in this study was found within this range.²⁶ The obtained variations in isolation rates may be related to plant conditions, processing procedure, geographical location, seasonal differences, experimental designs and analytical methods used to analyze the collected samples.¹²,²⁶ As an example, Levican and colleagues suggested that different conditions used for culturing can lead to different isolation rates. They found that the recovery rate of *Arcobacter* under aerobic incubation was higher (41.1%) than that of microaerobic conditions (23.2%).

*Arcobacter* spp. has been reported to be as a significant hazard for the public health.²⁶,²⁷ Their presence in food processing environments indicates possible persistence or cross-contamination.²⁸ The occurrence in poultry carcasses can be due to fecal contamination²⁹ or other possible transmission routes during handling (water, processing environment, equipment, feathers) or early stages of processing.³⁰-³² In fact, *Arcobacter* spp. can persist and form biofilms on many pipe surfaces made of steel, copper and polyethylene resulting in colonization in water distribution systems.³³ In a recent study, processing water and equipment used in the slaughterhouse were suggested as the main sources of broiler carcass contamination by *Arcobacter*.³⁴

The isolated *Arcobacter* species were further examined by m-PCR to identify at the species level. Based on the m-PCR, the most prevalent species were *A. butzleri* (n=45), followed by *A. cryaerophilus* (n=2) and *A. skirrowii* (n=1). The remaining *Arcobacter* isolates (n=17) could not be identified using the available primers at the species level (Table 1). Collado and colleagues also showed that these three species were found in the water and shellfish most probably due to sewage outlets and fecal contamination. In accordance with the results of previous studies,¹³,²¹ *A. butzleri* was the most prevalent species on chicken meat. *A. butzleri* was isolated from all types of samples analyzed. Its isolation rates from wings, breasts, drumstickers, carcasses, legs, breasts, and quarters were 100, 87.5, 84.6, 80, 76.9, and 72.7%, respectively. On the other hand, *A. cryaerophilus* and *A. skirrowii* were detected on the wings and carcass samples and on the drumsticker, respectively.

In eight analyzed samples, both *A. butzleri* and other two species were isolated. Similar to this finding, the presence of more than one species in the same sample was reported by other researchers.¹⁰,³¹ It has been suggested that lower isolation rates for *A. skirrowii* may be due to its higher susceptibility to antimicrobials present in selective media used or growth competition favoring other microorganisms.³⁶ *A. butzleri* has been found to cause enteritis and bacteremia in human³⁷ and *A. cryaerophilus* and *A. skirrowii* has been isolated from stool of patients with diarrhea.³⁸-⁴⁰ Since *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* are emerging foodborne pathogens, effective control methods should be applied to prevent *Arcobacter* spp. contamination during food processing for human health.

**Conclusions**

Based on the results, the presence of *Arcobacter* spp. in chicken meat is a potential risk for human health because the consumption of these contaminated products may cause serious diseases. Therefore, effective control strategies should be applied to prevent their contamination during poultry processing in the food industry.

**Table 1. Distribution of *Arcobacter* spp. based on the type of samples.**

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Samples analyzed</th>
<th>Positive samples (%)</th>
<th><em>A. butzleri</em> (%)</th>
<th><em>A. cryaerophilus</em> (%)</th>
<th><em>A. skirrowii</em> (%)</th>
<th>Non-identified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg</td>
<td>40</td>
<td>13 (32.5)</td>
<td>10 (76.9)</td>
<td>-</td>
<td>-</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Leg quarter</td>
<td>17</td>
<td>11 (64.7)</td>
<td>8 (72.7)</td>
<td>-</td>
<td>-</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Drumsticker</td>
<td>16</td>
<td>13 (81.3)</td>
<td>11 (84.6)</td>
<td>-</td>
<td>1 (7.7)</td>
<td>3 (23.1)</td>
</tr>
<tr>
<td>Breast</td>
<td>11</td>
<td>8 (72.7)</td>
<td>7 (87.5)</td>
<td>-</td>
<td>-</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Wing</td>
<td>10</td>
<td>5 (50)</td>
<td>5 (100)</td>
<td>1 (20)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carcass</td>
<td>6</td>
<td>5 (83.3)</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td>-</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>55 (55)</td>
<td>45 (80)</td>
<td>2 (3.6)</td>
<td>1 (1.8)</td>
<td>17 (30.9)</td>
</tr>
</tbody>
</table>
References


