Isolation of Arcobacter spp. from Retail Meats and Cytotoxic Effects of Isolates against Vero Cells


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ABSTRACT

A survey of Arcobacter spp. was conducted over a 12-month period in Guadalajara, Mexico. A total of 135 samples (45 lean ground beef samples, 45 lean ground pork samples, and 45 chicken samples, including drumsticks, gizzards, and ground or chopped breast) were collected from local butcheries. The samples were enriched in Johnson-Murano enrichment medium and then streaked onto Johnson-Murano agar plates. Typical colonies were subjected to microscopic and biochemical identification followed by polymerase chain reaction confirmation of the genus Arcobacter. All isolates confirmed to be Arcobacter isolates were then inoculated into Eagles’ minimum essential medium to determine their cytotoxicity against Vero cells. Arcobacter spp. were detected in 28.8, 51.1, and 40.0% of beef, pork, and chicken samples, respectively. From these samples, 101 isolates were confirmed to be Arcobacter by polymerase chain reaction. Overall, the species most frequently identified was A. butzleri, followed by A. skirrowii. A. cryaerophilus was isolated only from pork meat. Ninety-five (95%) of the Arcobacter isolates produced a virulence mechanism against Vero cells, and 38 of them induced cell elongation, indicating enterotoxin production. Eighteen isolates produced the formation of vacuoles, and 39 produced both vacuolization and elongation. The vacuolization effect may be related to a vacuolizing toxin. The production of a vacuolizing toxin by Arcobacter spp. has not previously been reported. Results obtained in this study indicate that Arcobacter spp. may show cytotoxic effects other than the recognized enterotoxin production.

Arcobacter was isolated in 1977 from aborted cow fetuses. Ellis et al. (4) described it as a Vibrio-like microorganism. Initially, these microorganisms were named Campylobacter cryaerophilus because of their similarities to Campylobacter spp. and their ability to grow at low temperatures. In 1992, Vandamme et al. (26) proposed the creation of a new genus for these aerotolerant campylobacters and named it Arcobacter. Four species, Arcobacter butzleri, Arcobacter skirrowii, Arcobacter nitrofigillus, and Arcobacter cryaerophilus, have been described for this genus. A. cryaerophilus is divided into two hybridization groups, groups 1A and 1B.

Although the ecology and the epidemiology of the infections caused by Arcobacter are not entirely known, food and water are important vehicles for this organism. In one study, Arcobacter spp. were recovered from about 80% of chicken and pork samples (18), and water has been identified as the vehicle for the organism in incidents of gastroenteritis (14).

The real impact of Arcobacter on public health remains largely unknown, because this organism is not usually investigated on a routine basis in clinical or industrial analyses. However, a few reports indicate that this organism may pose a relatively high risk for some populations (24, 30). A. butzleri has been isolated from patients with gastroenteritis (16, 29) and, in a few cases, from the blood of patients with liver or other systemic diseases (10, 22, 31). In contrast, A. cryaerophilus has been isolated only from patients with bacteremia (31). This information indicates that different species or strains may possess different virulence mechanisms. However, there are also very few reports on virulence factors for Arcobacter. Musmanno et al. (19) tested the cytotoxic effects of various strains of A. butzleri isolated from surface water in Italy against Intestine 407, CHO, HeLa, and Vero cell lines. These authors reported that 17 of 18 strains produced a cytotoxic effect against CHO and Vero cells, and one strain induced a cytotoxic-like effect on CHO cells and showed a capacity for adhesion to HeLa cells. In another study, Fernandez et al. (5) used the rat ileal loop test to demonstrate the enterotoxic effects of two strains of A. cryaerophilus, and these authors also noted an invasive effect of the same two isolates against 18-h-old Hep-2 cell cultures.

The first enriched medium used for recovering Arcobacter was Ellinghausen-McCullough-Johnson-Harris polysorbate 80 (EMJH-P80), a semisoloid medium originally designed for Leptospira (4). Although this medium is still used by some researchers (18, 25), other enrichment media have been designed specifically to enhance the isolation of Arcobacter. Among these media, Johnson-Murano enrichment medium (JMEM) and Johnson-Murano agar (JMA) were designed to permit the growth of Arcobacter under...
aerobic conditions, in contrast to other media that require a microaerophilic atmosphere. On JMA, *Arcobacter* produces round, translucent to grayish, shiny colonies with diameters of 1 to 2 mm (11, 12). Because these characteristics make the selection and differentiation of *Arcobacter* colonies easy, a laboratory method involving JMEM and JMA was used in this study. The objective of this work was to determine the frequency of *Arcobacter* spp., and, more specifically, *A. butzleri*, in samples of retail beef, pork, and chicken sold in Guadalajara City, Mexico, as well as to determine the ability of *Arcobacter* isolates to develop cytotoxic activity against Vero cells.

**MATERIALS AND METHODS**

**Sample collection and preparation.** One hundred thirty-five beef-pork or chicken butcher shops in the Guadalajara area were randomly selected. Over a period of 12 months, each shop was sampled once. With this sampling plan, 135 samples were collected, 45 each of ground beef, ground pork, and chicken (15 drumsticks, 15 edible viscera, and 15 ground or chopped breast samples). Each sample, weighing between 125 and 250 g, was placed inside a stomacher bag together with its original wrapping, sealed with a rubber band, and transported to the laboratory in a refrigerated container (25 × 15 cm; Igloo, Houston, Tex.) for analysis within 1 h after its collection.

**Isolation of *Arcobacter.*** A 25-g portion of each sample was deposited in a sterile stomacher bag with 225 ml of sterile 0.1% peptone water and pummeled for 1 min in a Stomacher 400 mixer (Lab-Blender; Tekmar Co., Cincinnati, Ohio). One milliliter of the resulting homogenate was transferred to each of two screw-cap tubes (16 by 150 mm) containing 10 ml of JMEM. These tubes were then incubated at 30°C for 48 h under an external aerobic atmosphere with the caps tightened. From this enrichment, three loopfuls were streaked onto each of two plates of JMA. These loopfuls were collected from points 0.5 to 1 cm beneath the surface of the medium as recommended by Johnson and Murano (12). The plates were incubated at 30°C for 48 h under aerobic conditions.

Three to five typical colonies (round, translucent to grayish, and shiny with diameters of 1 to 2 mm) were streaked onto a fresh plate of JMA to ensure a pure culture and were then subjected to *Campylobacter* modified Gram stain and oxidase, catalase, and motility tests (13, 28). Isolates consisting of gram-negative curved or helical rods that were catalase and oxidase positive were further tested according to the characteristics described by Harmon and Wesley (8) and 2 (2.0%) were identified as *A. cryaerophilus*. *A. cryaerophilus*, *A. skirrowii*, and *A. butzleri* were identified on the basis of the production of a red color in the vacuoles, which indicated a low pH.

**RESULTS**

According to the data in Table 1, 54 (40.0%) of the 135 raw meat samples analyzed in this study tested positive for *Arcobacter* spp. The type of meat with the highest percentage of isolation was pork (51.1%), followed by chicken (40.0%) and beef (28.9%). The 45 samples of chicken meats included 15 samples of drumsticks, 15 samples of edible viscera, and 15 samples of ground breast, and the frequencies of positive samples for these poultry products were 60, 42.9, and 20.0%, respectively. Data analysis indicated a significant difference (*P* < 0.05) between the percentage of positive samples for drumsticks and that for breast samples.

One hundred nine isolates obtained from these positive samples were presumed to be *Arcobacter* isolates on the basis of biochemical tests. Of these isolates, 101 were confirmed to be *Arcobacter* isolates by DNA analysis with PCR (8). Eighty (79.2%) of these isolates were identified as *A. butzleri*, 19 (18.8%) were identified as *A. skirrowii*, and 2 (2.0%) were identified as *A. cryaerophilus*. *A. cryaerophilus* was isolated only from pork.

*Arcobacter* spp. were seen to produce two effects in our study: a cytotoxic effect (cell elongation), indicating enterotoxin production, and vacuole formation, indicating the probable production of vacuolizing toxin (Fig. 1). Cells showing vacuole formation were treated with neutral red to
<table>
<thead>
<tr>
<th>Type of meat</th>
<th>No. of samples positive</th>
<th>% of samples positive</th>
<th>Species</th>
<th>No. of isolates</th>
<th>% of subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork ((n = 45))</td>
<td>23</td>
<td>51.1</td>
<td><em>A. butzleri</em></td>
<td>33</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. skirrowii</em></td>
<td>4</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. cryaerophilus</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subtotal</td>
<td>39</td>
<td>100.0</td>
</tr>
<tr>
<td>Chicken(^a) ((n = 45))</td>
<td>18</td>
<td>40.0</td>
<td><em>A. butzleri</em></td>
<td>30</td>
<td>73.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. skirrowii</em></td>
<td>11</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subtotal</td>
<td>41</td>
<td>100.0</td>
</tr>
<tr>
<td>Beef ((n = 45))</td>
<td>13</td>
<td>28.9</td>
<td><em>A. butzleri</em></td>
<td>17</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. skirrowii</em></td>
<td>4</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subtotal</td>
<td>21</td>
<td>100.0</td>
</tr>
<tr>
<td>Total ((n = 135))</td>
<td>54</td>
<td>40.0</td>
<td><em>A. butzleri</em></td>
<td>80</td>
<td>79.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. skirrowii</em></td>
<td>19</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. cryaerophilus</em></td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>101</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\(^a\) Nine (60\%) of 15 drumsticks, 6 (40.0\%) of 15 edible viscera, and 3 (20.0\%) of 15 breast samples tested positive. Data analysis indicated significant differences \((P < 0.05)\) in the percentages of positive samples for drumsticks and breasts.

verify that the vacuole formation was due to a VTLF and not to opsonization. As indicated by the data in Table 2, 95 isolates produced at least one of these two effects against Vero cells; 38 (37.6\%) produced the cytotoxic effect, 18 (17.8\%) produced vacuolization, and 39 (38.6\%) produced both vacuolization and the cytotoxic effect. Only 6 isolates (6.0\%) failed to produce an effect on Vero cells. The production of a specific cytotoxic effect did not seem to be

![FIGURE 1. Effects of filtrates of *Arcobacter* spp. on Vero cells. (A) Cytoplasmic vacuolization; the arrow indicates a typical vacuole \((\times100)\). (B) Normal Vero cells \((\times100)\). (C) Cytotoxic effect; arrows indicate enlarged cells \((\times40)\). (D) Normal Vero cells; arrows indicate normal round cells \((\times40)\).](https://meridian.allenpress.com/jfp/article-pdf/66/8/1374/1675825/0362-028x-66_8_1374.pdf)
related to a particular species. However, of the isolates identified as *A. butzleri*, 41.2% produced a cytotonic effect only and 18.8% produced a vacuolizing effect only. Statistical analysis indicated that these percentages were significantly different (*P* < 0.05). No significant difference was observed between the proportion of isolates producing enterotoxin and the proportion producing vacuolizing toxin for *A. skirrowii* or *A. cryaerophilus*.

**DISCUSSION**

Reports on the prevalence of *Arcobacter* in foods are scant, probably owing to the relatively recent description of the genus. Collins et al. (2) isolated *Arcobacter* from 0 to 90% of ground pork samples collected at five meat-processing plants. The information provided by these authors makes possible an understanding of how pathogens are introduced to meat during processing at the plant level. In contrast, our study on retail meats might provide information on other mechanisms of contamination, such as environmental contamination or cross-contamination. In addition, it was obvious during sampling at the butcher shops that most of the time the meat was kept out of the refrigerator for long periods to ease sales operations. Such a practice can result in the growth of pathogens, which may increase the risk to the consumer. Poor storage conditions such as those involving temperature abuse and keeping beef and pork together on the counter may also allow cross-contamination to occur, spreading microorganisms over noncontaminated meats. This might have been the reason we found a relatively high incidence (40.0%) of *Arcobacter* in retail meats, with the highest incidence being that for ground pork, whereas others have reported lower frequencies of isolation from pork. For example, De Boer et al. (3) isolated *Arcobacter* from 24.1% of retail poultry samples and from only 1 (1.5%) of 68 samples of minced beef and 1 (0.5%) of 194 samples of minced pork. Reports on the isolation of this organism from pork indicate that the prevalence varies from 3.7% to as high as 90%, with variations depending on the processing plant and even the day of sampling (2, 8). Several authors have found the prevalence of *Arcobacter* in poultry to be as high as 90 to 100% (1, 7–9, 11, 15, 27, 29). In the present study, *Arcobacter* spp. were recovered from 18 (40%) of 45 samples of chicken meats. *A. butzleri* has been reported to be the *Arcobacter* species most commonly isolated from chicken (17), a finding that is consistent with our results. Further research involving a larger number of samples tested over a longer period will determine seasonal variation in the prevalence of *Arcobacter* in retail meats.

Reports regarding *Arcobacter* virulence mechanisms are also limited. Despite the close relationship between *Arcobacter* and the genus *Campylobacter*, there are some differences in the possible cytotoxic effects of these two genera. Musmanno et al. (19) reported that while some *Campylobacter* isolates are able to produce a toxin that is responsible for the enlargement and death of cells in vitro (a cytolethal distending effect), no cytolethal distending activity was observed for *Arcobacter*. In contrast to the results of the present study, these authors did not detect any vacuolizing effects for *Arcobacter*, while over half of our isolates produced vacuoles, either as a single effect or in combination with cell elongation. These discrepancies may be due to differences in the incubation time elapsing prior to microscopic cell examination. Musmanno et al. (19) conducted cell examination after 24 h of incubation, whereas in our work the cell plates were examined after 7 h of incubation. In our laboratory experience, we have observed that the incubation time for as long as 24 h may result in cellular lysis, which makes it difficult to determine whether or not a vacuole has been formed. In addition, we tested a relatively large number of isolates, which may have increased the chances of detecting unknown or rare characteristics (such as the induction of vacuoles) among isolates. The production of vacuolizing toxin by *Arcobacter* spp. has not been reported. Organisms such as *V. cholerae* non-O1 and *Helicobacter pylori* have been reported to produce a cytotoxin that induces the formation of acid vacuoles in the cytoplasms of different cell lines. This toxin was named VacA and was described by Leunk et al. in 1988 (16). The production of a VTLF by some of our isolates may be indicative of VacA production by *Arcobacter*.

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