Study on Prevalence of Arcobacter spp. in Human and Foods of Animal Origin


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ABSTRACT

Arcobacters are considered potential zoonotic emerging food and waterborne pathogens. In the present study, a total of 205 samples of diverse origin comprising chicken meat (104), pork (51) and human stool (50) samples were collected from Bareilly region. A total of 65 samples were found positive for Arcobacter species by genus specific PCR method targeting 16S rRNA, giving an overall prevalence of 31.7%, wherein chicken meat samples showed 39.42%, pork 37.25% and human stool samples 10% prevalence. By conventional cultural method prevalence was found to be 23.41%, where pork samples showed higher prevalence (33.33%), followed by chicken meat samples (28.85%), and human stool samples (2%). Application of multiplex PCR for Arcobacter isolates targeting 16S and 23S rRNA genes for species identification revealed prevalence of A. butzleri (39.58%), A. skirrowii (2.08%), A. cryaerophilus (25%), both A. butzleri and A. cryaerophilus (14.58%), both A. butzleri and A. skirrowii (6.25 %) and both A. skirrowii and A. cryaerophilus (6.25%) and all the three species (6.25%). High prevalence rate of Arcobacter spp., in food samples of animal origin has important public health implications.

Keywords: Arcobacter, chicken meat, isolation, pork, prevalence

Introduction

Arcobacter belongs to the family Campylobacteriaceae. Arcobacters were first isolated from aborted bovine fetuses and later from porcine fetuses (Ellis et al., 1977, 1978). They are Gram-negative, non-spore forming, spirally curved to 'S'-shaped rods showing a cork-screw or darting type of motility, and formerly known as 'aerotolerent Campylobacters' (Vandamme and De ley, 1991). Arcobacter can grow microaerobically and aerobically and has the ability to grow at 15°C and 30°C, which is a distinctive feature that differentiates Arcobacter species from Campylobacter species (Vandamme and De ley, 1991 and Lehner et al., 2005). There is increase in number of reports on Arcobacter during the recent years so Arcobacter spp. has gained increased attention as emerging foodborne entero-pathogen.

Three species, A. butzleri, more rarely A. skirrowii and A. cryaerophilus, have been associated with human enteritis and occasionally bacteraemia (On et al., 1995; Ingrid et al., 2004; Lehner et al., 2005). Kownhar et al. (2007) reported 3 Arcobacter spp. and 16 Campylobacter jejuni, from 200 HIV infected patients with diarrheal symptoms in southern India. A. butzleri and A. cryaerophilus have been associated with incidences of human diarrhea and possibly septicaemia (Mansfield and Forsythe, 2000). Investigation revealed that A. butzleri and A. cryaerophilus are commonly present on slaughter equipment (Vytrasova et al., 2003). Foods of animal origin, such as poultry and pork meat are also routinely contaminated by Arcobacter spp. (Philips, 2001). Recently, Collado et al. (2009) reported that
the global prevalence of *Arcobacter* in different food samples was 32%; it was highest in clams (100%) and chicken (64.3%), followed by pork (53.0%), mussels (41.1%), duck meat (40.0%), turkey meat and beef had a similar recovery rate (33.3% and 31.3%; respectively), and rabbit meat had showed no prevalence.

In view of culture failure and misidentification, nucleic acid approaches, particularly polymerase chain reaction (PCR)-based methods, are increasingly being considered for detection, identification and monitoring of arcobacters in foods of animal origin, clinical samples of both animals and humans. Both genus specific and species specific PCR have been developed for rapid detection of arcobacters. (Harmon and Wesley, 1996; Houf et al., 2000). Furthermore, additional information on the epidemiology of these microorganisms is also necessary. Thus, taking into account all the points discussed above, the present study was undertaken to estimate the prevalence of *Arcobacter* spp. in human and foods of animal.

**Materials and Methods**

A total of 205 samples of diverse origin viz., chicken meat samples (104) and pork (51) were collected aseptically in plastic containers from various retail shops of Bareilly, pig slaughter house Bareilly region. Human stools swabs (50) were collected aseptically in PBS (10% w/v), from human hospital IVRI, nursery and other private clinics in and around Bareilly. The samples were transported on ice pack and processed within 24-48 h of collection.

Ten gram of chicken and pork samples were aseptically minced with scissors and suspended in 90 ml of PBS. The mixtures were homogenized with stomacher for 1 min at 200 rpm. 1 ml of suspension was inoculated into 10 ml of CAT broth (*Arcobacter* broth base supplemented with cefoperazone, amphotericin B and teicoplanin) with 5% defibrinized sheep blood and incubated at 30°C under microaerophilic condition (5% O₂, 10% CO₂ and 85% N₂) for 48 h. After homogenization of human stool swabs sample, 1ml of the fecal suspension was inoculated in to 9 ml CAT broth with 5% defibrinized sheep blood in 1:9 ratio and incubated at 30°C for 48 h under micro-aerophilic condition.

After enrichment, whole cell DNA was extracted from all the samples by heat lysis (snap chill) method and were subjected to optimized protocols of genus specific PCR for *Arcobacter* spp. confirmation targeting 16S rRNA gene using primers ARCO-I and ARCO-II (Harmon and Wesley, 1996).

**Conditions used for genus specific PCR**

For the detection of Genus *Arcobacter*, PCR-protocol was optimized using primer set Arco-I (5'-AGAGATTAGCTGTATTGTAT-3') and Arco-II (5'-TAGCATCCCCGCTTCGAATGA-3'), which were designed from 16S rRNA genes by Harmon and Wesley (1996) with the expected product size 1223 bp (Fig. 1). In a total of reaction mixture, 2.5 µl of 10X PCR DreamTaq buffer, 1.5 µl of 2 mM dNTP, 1.5 µl of each ARCO-I and ARCO-II primers (15 pmol), 0.2 µl of Taq Polymerase (1U), 4 µl of template DNA and nuclease free water to make final volume of 25 µl. PCR-cycling were performed with initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 51°C for 30 sec and extension at 72°C for 1 min. Final extension was done at 72°C for 10 min. After the reaction cycles, the PCR product was electrophoresed on 1.5% agarose gel and analyzed by using UV trans-illuminator (Gel-Doc system).

Genus specific PCR positive samples were filtered using 0.45 µm pore size PES syringe filter directly on to CAT agar plates and incubated under aerobic condition at 30°C for 48-72 h. Isolates showing typical colony characters and morphology i.e. translucent to whitish, 2-4 mm in diameter, round and convex colonies were subjected for biochemical characteristics. DNA from these colonies was isolated by heat lysis method and multiplex PCR assay was applied targeting 16S rRNA gene for detection of *A. butzleri, A. skirrowii* and 23S rRNA gene for *A. cryaerophilus* using primers developed by Houf et al. (2000).

**Conditions used for species specific PCR**

Detection of *Arcobacter* spp. (*A. butzleri, A. cryaerophilus*, and *A. skirrowii*) was performed
using primer sets BUTZ, ARCO, SKIR, CRY-1, and CRY-2; which were designed from 16S rRNA and 23S rRNA genes by Houf et al. (2000), with slight modification of the original protocol. A band size of 401 bp, 257 bp and 641 bp obtained for A. butzleri, A. cryaerophilus and A. skirrowii, respectively (Fig. 2).

The 25 µl reaction mixture was composed of 2.5 µl of 10x PCR buffer; 1 U of Taq DNA polymerase; 0.2 mM of each deoxyribo nucleotide triphosphate, 2.5 mM MgCl₂, 15 pmol of the primers ARCO, BUTZ, CRY-1, CRY-2 and 7.5 pmol of primer SKIR; 5 µl heat lyses DNA of the bacteria as template and the final volume was adjusted to 25 µl with nuclease free water. Cyclic conditions involved an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (94°C for 30 sec.), primer annealing (51°C for 30 sec) and chain extension (72°C for 1 min) and final extension at 72°C for 10 min. The PCR product was electrophorased on 1.5% agarose gel and analyzed by using UV trans-illuminator.

Results and Discussion

A total of 65 samples were found positive for Arcobacter species by genus specific PCR method, giving an overall prevalence of 31.7%, wherein chicken meat showed 39.42%, pork 37.25% and human stool 10% prevalence (Table 1). By conventional cultural method 48 isolates were isolated giving an overall prevalence of 23.41%, where pork samples showed higher prevalence (33.33%), followed by chicken meat (28.85%) and human stool samples (2%).

Table 1. Number of samples detected positive by genus specific PCR and cultural method

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Type of samples</th>
<th>Number of samples found positive by gPCR</th>
<th>Number of samples found positive by cultural method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human stool samples</td>
<td>5 (10%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>2</td>
<td>Chicken meat</td>
<td>41 (39.42%)</td>
<td>30 (28.85%)</td>
</tr>
<tr>
<td>3</td>
<td>Pork</td>
<td>19 (37.25%)</td>
<td>17 (33.33%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>65 (31.7%)</td>
<td>48 (23.41%)</td>
</tr>
</tbody>
</table>
Table 2. Different Arcobacter species detected by m - PCR from colonies

<table>
<thead>
<tr>
<th>Type of Samples</th>
<th>Total positive detected</th>
<th>Only A. butzleri</th>
<th>Only A. skirrowii</th>
<th>Only A. cryaerophilus</th>
<th>A. butzleri and A. skirrowii</th>
<th>A. butzleri and A. cryaerophilus</th>
<th>A. skirrowii and A. cryaerophilus</th>
<th>A. butzleri and A. skirrowii and A. cryaerophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human stool samples</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Foods of animal origin</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Chicken</td>
<td>30</td>
<td>12</td>
<td>01</td>
<td>07</td>
<td>02</td>
<td>02</td>
<td>05</td>
<td>01</td>
</tr>
<tr>
<td>Pork</td>
<td>17</td>
<td>06</td>
<td>—</td>
<td>05</td>
<td>01</td>
<td>01</td>
<td>02</td>
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<td></td>
<td>48</td>
<td>19</td>
<td>01</td>
<td>13</td>
<td>03</td>
<td>03</td>
<td>07</td>
<td>03</td>
</tr>
<tr>
<td>(39.58%)</td>
<td>(2.08%)</td>
<td>(25%)</td>
<td>(6.25%)</td>
<td>(6.25%)</td>
<td>(14.58%)</td>
<td>(6.25%)</td>
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</table>

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References


The result of this study indicated that PCR based methods are better for detection compared to cultural isolation technique (Table 1). A. butzleri has shown higher prevalence in the present study. Since A. butzleri has been reported for higher resistance to antibiotics and the prevalence of Arcobacter spp. was found highest in poultry (62%), followed by pork (35%) and human stool samples (10%) of Arcobacter spp. were found to be common contaminants of retail raw meats in Northern Ireland. Molecular diagnostics optimized in present study helped in identification of different species of Arcobacter. Co-colonization of single host with more than one species is quite common.


