Prevalence of *Arcobacter* spp. in Humans, Animals and Foods of Animal Origin in India Based on Cultural Isolation, Antibiogram, PCR and Multiplex PCR Detection

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ABSTRACT

*Arcobacter* is an important emerging food and water borne pathogen having worldwide public health concern. The present study reports the prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin based on cultural isolation, antibiogram, Polymerase Chain Reaction (PCR) and multiplex PCR detection. A total of 400 samples were collected as human diarrheal stool (50), faecal swabs of poultry (50), pig (50), cattle (50) and foods of animal origin [Raw milk (60), chicken meat (60), beef (40) and pork (40)]. The overall prevalence rate of *Arcobacter* spp. was found to be 6.78% (27/400) by cultural isolation with highest prevalence in pig faeces (12%), followed by cattle faeces (10%), chicken meat (10%), poultry faeces (8%), beef (5%), pork (5%), human diarrheal stools (2%) and milk (1.67%). PCR screening revealed prevalence of *Arcobacter* spp. to be 7.75% (31/400) with highest in pig faeces (12%), followed by cattle faeces (12%), chicken meat (11.67%), poultry (10%), beef (7.5%), pork (5%), human stools (2.00%) and raw milk (1.67%). Multiplex PCR assay enabled detection of *A. butzleri* (21/27) and *A. skirrowii* (6/27). In vitro antibiotic sensitivity profile of 27 *Arcobacter* isolates revealed most of these to be sensitive to azithromycin, gentamycin, nalidixic acid, kanamycin, streptomycin, ciprofloxacin and tetracycline. Higher resistance was observed for cephalothin, novobiocin and vancomycin with notable intermediately resistance against erythromycin and chloramphenicol. The present study demonstrated high prevalence of *Arcobacter* spp. in pig, cattle and poultry faecal samples which may play important role in contamination of environment, water and human food chain, thus could be of public health concerns. The PCR was found to be more rapid, sensitive, specific and efficient than cultural methods for detection of *Arcobacter* spp.

Key words: *Arcobacters*, prevalence, humans, animals, foods, meat, milk, cultural isolation, antibiogram, PCR, multiplex PCR

INTRODUCTION

*Arcobacters* are fastidious, microaerophilic, non-sporing, motile, spiral-shaped organisms, 0.2-0.9 μm wide by 1-3 μm long and were formerly known as aerotolerant campylobacters
Arcobacters were first isolated from aborted bovine fetuses and later from porcine fetuses (Ellis et al., 1977, 1978). Culturally, Arcobacter can grow microaerobically by evacuating 80% of the normal atmosphere and introducing a gas mixture of 8% carbon dioxide, 8% hydrogen and 84% nitrogen into the jar and aerobically at 15 and 30°C (Vandamme and De Ley, 1991). Arcobacters show respiratory type of metabolism, non-saccharolytic, oxidase and catalase positive nature. The genus Arcobacter though comprised of eighteen species but A. butzleri, A. cryaerophilus, A. skirrowii, A. cibarus are considered as important emerging food-borne pathogens (Figueras et al., 2012; Levican and Figueras, 2013; Levican et al., 2013).

Arcobacters have been isolated from healthy cattle, sheep, horses and more frequently in pigs (Lehner et al., 2005; Ho et al., 2006a, b). These are also known to be associated with various disease conditions in animals such as mastitis, diarrhea, abortion and reproductive disorders in cattle, sheep and swine (On et al., 2002; Lehner et al., 2005; Ho et al., 2006a, b; Snelling et al., 2006). In humans, primarily A. butzleri has been found to be associated with enteritis while A. cryaerophilus and A. skirrowii have been isolated from stool of diarrheic patients (Vandenberg et al., 2004; Wybo et al., 2004; Same et al., 2007).

Among the foods of animal origin, Arcobacters have been isolated from chicken meat, milk, pork and seafood (Rivas et al., 2004; Patyal et al., 2011; Bagalakote et al., 2013; Ramees et al., 2014a, b). Arcobacters have worldwide distribution and have been reported from US, Denmark, Brazil, Japan, Australia, The Netherlands, Belgium, Spain and Italy, Czech Republic, Korea, Malaysia and others from a variety of sources (Lehner et al., 2005; Atabay et al., 2006; Pejchalova et al., 2008; Collado et al., 2010; Amare et al., 2011). Contaminated drinking water has been identified as a major source of infection in developing countries (Taylor et al., 1991), though the manipulation, consumption and cross contamination of raw and undercooked meat products are more likely cause of infection in industrialized countries (Lehner et al., 2005; Ho et al., 2006a; Snelling et al., 2006). Recently, close contact with pets has been suggested as another potential infection source for humans (Pera et al., 2009; Houf et al., 2008).

In comparison to the important food-borne pathogens like Salmonella, Campylobacter, Escherichia spp. and others the emergence and evolution Arcobacters has been recognized especially during last two decades (Lee et al., 2010; Vilar et al., 2010; Amare et al., 2011). Arcobacter spp. contamination in fresh vegetables has also been reported only recently (Gonzalez and Ferrus, 2011). There is evidence that healthy livestock animals may be a significant reservoir of Arcobacter spp. Based on the many a reports of the presence of Arcobacter spp. from animals and humans including various food, raw milk and water sources, Arcobacters have been considered to possess potential zoonotic implications (Ho et al., 2006a; Snelling et al., 2008; Scullion et al., 2003). Also, many Arcobacter spp. isolated from humans, chicken carcasses, meat and environmental sources were found to be resistant to antimicrobials commonly used in human and veterinary medicine (Houf et al., 2001a; Kabeya et al., 2004). Information on the presence of arcobacters in various sources in India is limited, so the present study was undertaken to determine the occurrence of Arcobacter spp. in humans, animals and foods of animal origin.

**MATERIALS AND METHODS**

**Sample collection:** In this study, a total number of 400 samples were collected from various sources including of faecal swab samples of animals (poultry, pigs, cattle) (50 each) from pig and poultry farms; diarrheal stool samples of humans (50) from human hospitals and food samples (200) of animal origin like raw milk (60) from dairy farms, chicken meat (60), beef (40) and pork (40).
Table 1: Prevalence of *Arcobacters* in different samples using conventional cultural and modern molecular tool of PCR

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Source</th>
<th>No. of samples</th>
<th>No. of samples detected positive for <em>Arcobacter</em> spp.</th>
<th>Conventional cultural methods</th>
<th>Standardized genus based PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human diarrhoeal stool</td>
<td>Human hospital, Indian Veterinary Research Institute (IVRI), Izatnagar and different Private Hospitals, Izatnagar, Bareilly (Uttar Pradesh)</td>
<td>50</td>
<td>1</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Poultry</td>
<td>Poultry farms, Central Avian Research Institute (CARI), Izatnagar and retail chicken market, Bareilly (Uttar Pradesh)</td>
<td>50</td>
<td>4</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pig</td>
<td>Pig farm, IVRI, Izatnagar, Bareilly (Uttar Pradesh)</td>
<td>50</td>
<td>6</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Cattle</td>
<td>Dairy farm, IVRI, Izatnagar, Bareilly (Uttar Pradesh)</td>
<td>50</td>
<td>5</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Food samples of animal origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw milk</td>
<td>Dairy farm, IVRI, Izatnagar, Bareilly (Uttar Pradesh)</td>
<td>60</td>
<td>1</td>
<td>1.07%</td>
<td>1</td>
</tr>
<tr>
<td>Chicken</td>
<td>Slaughter house, CARI, Izatnagar and chicken retail shops, Bareilly (Uttar Pradesh)</td>
<td>60</td>
<td>6</td>
<td>10%</td>
<td>7</td>
</tr>
<tr>
<td>Beef</td>
<td>Slaughter house, retail shops Bareilly (Uttar Pradesh)</td>
<td>40</td>
<td>2</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pork</td>
<td>Slaughter house, retail shops Bareilly (Uttar Pradesh)</td>
<td>40</td>
<td>2</td>
<td>5%</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>400</td>
<td>27</td>
<td>6.75%</td>
<td>31</td>
</tr>
</tbody>
</table>

from retail shops and slaughter houses in and around Bareilly (Uttar Pradesh, India) (Table 1). The samples collected from humans represented clinical cases of diarrhea while of poultry, pig and cattle were non-diarrheal (representing healthy animals). All the samples were processed for isolation and characterization of *Arcobacter* spp. using standard isolation and identification methods. The faecal swabs and stool samples were brought fresh immediately after collection in phosphate buffer saline (PBS, 10% w/v) while meat samples collected fresh were transported under refrigerated conditions to the laboratory under aseptic and sterile conditions. All the collected samples were examined in the laboratory within 24-48 h of their collection.

**Isolation and identification of *Arcobacters***: The *Arcobacter* selective isolation was carried out as described previously (Houf et al., 2001b). Briefly, after homogenization of faecal swab samples in 10 mL PBS (10% w/v), 1 mL of faecal suspension was inoculated in to 9 mL *Arcobacter* enrichment broth (1:9) (Houf’s broth) with selective supplement of Cefoperazone (15 mg L⁻¹), 5-fluorouracil (100 mg L⁻¹), Amphoteracin B (10 mg L⁻¹), Novobiocine (32 mg L⁻¹) and Trimethoprim (64 mg L⁻¹) and 5% defibrinated sheep blood for enrichment. Ten grams of meat samples were aseptically minced with scissors and suspended in 90 mL of PBS (10% w/v), homogenized with stomacher for 1 min at 200 rpm; 1 mL of suspension was inoculated into enrichment broth. Further incubation of all the respective enrichment broth tubes were performed at 28°C for 48 h under microaerophilic (5% O₂, 10% CO₂ and 85% N₂) conditions for enrichment of *Arcobacters*. These were then streaked onto *Arcobacter* selective agar plates with antibiotic supplements, incubated microaerobically for 48-72 h. The suspected *Arcobacter* colonies (typical translucent to whitish, round and convex) were tested by gram's staining, cellular morphology, catalase and oxidase test and for motility using wet mount method. Upto 4-5 colonies were picked from each agar plate and subcultured on *Arcobacter* blood agar plates [*Arcobacter* broth (Oxoid),
Agar Technical No. 3 (Oxoid) fortified with 5% defibrinated sheep blood without selective antibiotic supplements and incubated aerobically at 28°C for 48 h. A single colony was selected from the blood agar plates for microscopic examination and biochemical tests (Vandamme and De Lay, 1991; Harrab et al., 1998).

Reference strain DNA and DNA extraction from *Arcobacter* isolates: For control studies, the positive DNA of *Arcobacter butzleri* (LMG 10828^T^) was procured from Belgium Bacterial Collection (BCCM/LMG). The genomic DNA from presumptive *Arcobacter* colonies and from enrichment broths were isolated by snap chill method. In brief, an aliquot of each isolate was prepared in 1 mL of sterile water and centrifuged for 5 min at 14,000 rpm to pellet the cells. The supernatant was discarded, pellet was resuspended in 100 μL of sterile water and heated for 10 min in a boiling water bath to lyse the cells. These were centrifuged for 5 min to pellet cell debris and 5 μL of supernatant (lysate) was used as the DNA template in the genus and multiplex (MP) Polymerase Chain Reaction (PCR) assay. Purity and concentration of DNA was checked by 0.8% agarose gel electrophoresis and nano drop technique (Nanodrop, USA).

Genus level detection by Polymerase Chain Reaction (PCR): The confirmation of the suspected *Arcobacter* isolates was carried out by genus specific-PCR as described previously (Harmon and Wesley, 1996) with some modifications. In brief, Arco-I and Arco-II primer set was used for 16S rRNA gene amplification with predicted product size of 1233 bp. Briefly, to 5 μL of 10X Taq buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 10 pmol of each Arco-I and Arco-II primers, 2.5 U Taq polymerase, 5 μL of heat-lyses bacterial DNA or 20 ng of purified DNA as template and nuclease free water was added to make total volume to 50 μL of reaction mixture. Amplification of template DNA was carried out by PCR-cycling 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. The amplified PCR products were electrophoresed on 1% agarose gel and analyzed by using UV trans-illuminator. Specificity of standardized PCR assay and primers were checked with other common intestinal bacterial DNA (*C. jejuni*, *C. coli*, *Salmonella*, *Aeromonas* and *E. coli*).

Multiplex PCR assay: For differentiation at species level, all the *Arcobacter* isolates which were found positive by genus based-PCR were subjected to *Arcobacter* spp. specific multiplex-PCR (MP-PCR) as described previously (Houf et al., 2000). Published primer sets viz., BUTZ, ARCO, SKIR, CRY-1 and CRY-2, designed for 16S rRNA and 23S rRNA genes, were employed for the identification of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. The DNA of *A. butzleri* (LMG 10828^T^) was used as positive control. The reaction mixture comprised of 5 μL of 10X PCR buffer, 2.5 U of *Taq* DNA polymerase, 0.2 mM of each dNTP, 2.5 mM MgCl₂, 15 pmol of the primers (ARCO butt, CRY-1, CRY-2 and SKIR), 5 μL heat lysed bacterial DNA template in the final volume of 50 μL. The MP-PCR involved an initial denaturation (94°C/5 min) followed by 30 cycles of denaturation (94°C/30 sec), primer annealing (51°C/30 sec) and extension (72°C/1 min) and final extension at 72°C for 10 min. The PCR products were visualized by electrophoresis using 1.5% agarose gel and UV trans-illuminator.

Antibiogram: Antibiogram of *Arcobacter* spp. recovered in the present study was carried out against commonly used antibiotics as per the disc diffusion method (Bauer et al., 1966). The bacterial suspension of 48 h old culture of *Arcobacter* isolates were prepared in BHI broth and
incubated at 28°C. Test culture was inoculated onto the antibiotic test medium agar plates by using sterile cotton swabs. Plates were allowed to dry for 10 min. After drying the plates, antibiotic discs (Hi-media, India) were placed on agar plates by using sterile forceps. The plates were incubated at 28°C for 48 h in microaerophilic conditions for determining the susceptibility of bacterial isolates.

RESULTS
Prevalence of Arcobacters: In the present study, a total of 35 Arcobacter suspected colonies were recovered from the 400 different samples tested. The species specific prevalence of arcobacters in various test samples of humans, animals and foods of animal origin is tabulated in Table 1. Based on the cultural and biochemical analysis, a total of 27 (6.75%) presumptive Arcobacter species were finally isolated. The isolated colonies were suspected as Arcobacter due to white to whitish-gray, small (2-4 mm) diameter, convex and opaque with entire edge, smooth, transparent/ translucent characteristics. Furthermore, Arcobacter isolates (27) were Gram-negative, spirally curved rod or short “S” shape organisms, motile, oxidase and catalase positive, indoxyl acetate hydrolysis test and nitrate reduction positive and negative for the urease test, hippurate hydrolysis test and H₂S production from Triple Sugar Iron agar (TSI). These were sensitivity to nalidixic acid and resistance to cephalotin and showed cultural growth at 28°C aerobically but with no growth at 42°C, microaerobically. The highest prevalence was found in pig faeces (12%) followed by cattle faeces (10%), chicken meat (10%), poultry faeces (8%), beef (5%), pork (5%) human diarrheal stool (2%) and raw milk (1.67%).

Detection of Arcobacters by genus based-PCR: On application of standardized PCR assay, expected amplification product of 1223 bp size with positive A. butzleri DNA was produced. The assay was found to be highly specific for arcobacters detection as no other related bacterial DNA’s tested were amplified during the assay (Fig. 1). The PCR assay was found to be highly sensitive

Fig. 1: Specificity of genus based PCR assay for Arcobacter. Lane M: 100 bp DNA ladder, Lane 1: Arcobacter butzleri, Lane 2: C. Jejuni, Lane 3: C. coli, Lane 4: Salmonella typhimurium and Lane 5: Aeromonas spp.
in detecting *A. butzleri* DNA level to the extent of 1 pg μL⁻¹ concentration (Fig. 2). Application of PCR confirmed all the 27 culturally positive presumptive *Arcobacter* isolates to be positive (Fig. 3). Screening of all the collected samples (400) by PCR after enrichment revealed 31 out of 400 as

Fig. 2: Sensitivity of genus specific PCR assay for *Arcobacter*. Lane M: 100 bp DNA ladder, Lane 1: 0.1 pg μL⁻¹, Lane 2: 1 pg μL⁻¹, Lane 3: 10 pg μL⁻¹, Lane 4: 1×10² pg μL⁻¹, Lane 5: 1×10³ pg μL⁻¹, Lane 6: 1×10⁴ pg μL⁻¹, Lane 7: 5×10⁴ pg μL⁻¹

Fig. 3: Confirmation of *Arcobacter* isolates by genus based PCR. Lane M: 1 k bp DNA ladder, Lane 1: Positive control DNA of *Arcobacter butzleri* and Lane 2-6: Positive for genus *Arcobacter*
positive (7.75%) (Table 1). The highest rate of *Arcobacter* detection by PCR was from pig faeces (12%), followed by cattle faeces (12%), chicken meat (11.67%), poultry faeces (10%), beef (7.5%), pork (5%), human stools (2.00%) and raw milk (1.67%).

**Identification of Arcobacters at species level by multiplex PCR:** Application of standardized multiplex PCR assay for the 27 *Arcobacter* isolates indicated two different *Arcobacter* spp. with amplification product of 401 bp for *A. butzleri* and 641 bp for *A. skirrowii* (Fig. 4). Out of 27 isolates, 21 were *A. butzleri* and 6 were *A. skirrowii* (Table 2).

**Antibiogram studies:** Most of the *Arcobacter* isolates showed sensitivity to azithromycin, gentamycin, nalidixic acid, kanamycin, streptomycin, ciprofloxacin and tetracydine (100, 100, 100, 100, 100, 100, 100).

<table>
<thead>
<tr>
<th>Culturally positive <em>Arcobacter</em> isolates recovered from different samples</th>
<th><em>A. butzleri</em></th>
<th><em>A. skirrowii</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human diarrhoeic stool (1)</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Poultry faeces (4)</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Cattle faeces (5)</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Pig faeces (6)</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Raw milk (1)</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Chicken (6)</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Beef (2)</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Pork (2)</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

Fig. 4: Species level detection of *Arcobacter* isolates by multiplex PCR. Lane M: 1 k bp DNA ladder, Lane 1: Positive control DNA of *Arcobacter butzleri*, Lane 2, 4, 5 and 6: Positive for *Arcobacter butzleri*, Lane 3: Negative control and Lane 7: positive for *Arcobacter skirrowii*
Table 3: In-vitro antibiotic sensitivity profile of Arcobacter isolates

<table>
<thead>
<tr>
<th>Dose of antibiotics (µg)</th>
<th>Sensitive</th>
<th></th>
<th>Intermediate</th>
<th></th>
<th>Resistant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Azithromycin (30)</td>
<td>27</td>
<td>100.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Ciprofloxacin (5)</td>
<td>25</td>
<td>92.59</td>
<td>2</td>
<td>7.40</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Clindamycin (2)</td>
<td>22</td>
<td>81.48</td>
<td>5</td>
<td>18.52</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>2</td>
<td>7.40</td>
<td>25</td>
<td>92.59</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>27</td>
<td>100.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Nalidixic acid (30)</td>
<td>27</td>
<td>100.00</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>24</td>
<td>88.88</td>
<td>3</td>
<td>11.11</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Chloramphenicol (30)</td>
<td>0</td>
<td>0.00</td>
<td>24</td>
<td>88.88</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>Cephalothin (30)</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
<td>27</td>
<td>100.00</td>
</tr>
<tr>
<td>Novobiocin (30)</td>
<td>0</td>
<td>0.00</td>
<td>2</td>
<td>7.40</td>
<td>25</td>
<td>92.59</td>
</tr>
<tr>
<td>Kanamycin (30)</td>
<td>26</td>
<td>96.29</td>
<td>1</td>
<td>3.70</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Streptomycin (30)</td>
<td>26</td>
<td>96.29</td>
<td>1</td>
<td>3.70</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Vancomycin (30)</td>
<td>0</td>
<td>0.00</td>
<td>2</td>
<td>7.40</td>
<td>25</td>
<td>92.59</td>
</tr>
</tbody>
</table>

96.29, 96.29, 92.59 and 88.88%, respectively) as shown in Table 3. Higher resistance was observed for cephalothin, novobiocin and vancomycin antibiotics (100, 92.59 and 92.59%, respectively). Notable percentages of isolates were immediately resistant against erythromycin and chloramphenicol (92.59 and 88.88%, respectively) as shown in Table 3.

DISCUSSION

Arcobacters are emerging food-borne pathogens possessing public health concerns worldwide (De Smet et al., 2011; Dhama et al., 2013; Merga et al., 2013; Ramees et al., 2014a). Arcobacters have been isolated not only from normal healthy individuals and diseased animals but also from a variety of different food sources (Collado and Figueras, 2011; Patyal et al., 2011). However, reports regarding occurrence and prevalence of arcobacters in animals, humans and/or food sources from India are very scarce (Kownhar et al., 2007; Jiang et al., 2010; Patyal et al., 2011; Bagalakote et al., 2013; Ramees et al., 2014a, b). Therefore, the present study reports the prevalence of two Arcobacter spp. (A. butzleri and A. skirrowii) in pig faeces, cattle faeces, poultry faeces, chicken meat, beef, pork, milk and human diarrheal stool employing both conventional cultural methods as well as molecular techniques.

In the present study, the highest prevalence of Arcobacter spp. was found in pig faeces (12%), cattle faeces (10%), chicken meat (10%) and poultry faeces (8%), indicating their role as a major source of Arcobacter infections to human beings. The last decade saw many studies from various countries on the isolation and identification of Arcobacter spp. with variable prevalence rates reported from cattle, pigs, chickens, sheep, horses, dogs and humans and various food products especially meat of poultry, pork, lamb, beef (Van Driessche et al., 2004, 2005; Ho et al., 2006a, b; Prouzet-Mauleon et al., 2006; Houb and Stephan, 2007; Collado et al., 2009; Figueras et al., 2008; Amare et al., 2011; Bagalakote et al., 2013). Earlier studies reported the Arcobacter spp. from 3.3, 10 and 14.5% of the cattle and swine faecal samples and chicken cloacal swabs, respectively (Kabeya et al., 2003a). Also, Ongor et al. (2004) reported Arcobacter spp. in 9.5% of 200 rectal faecal samples collected from cattle in Turkey.

Arcobacters were most of time isolated from faecal samples as indicated by higher incidences in porcine, bovine faecal samples and gut samples of broilers (Wesley et al., 2000; Van Driessche et al., 2003; Ho et al., 2008). In dairy cows, prevalence of Arcobacter spp. was
assessed to be much higher (Wesley et al., 2000; Golla et al., 2002). In Denmark, from fresh droppings and cloacal swabs of poultry, a total of 85 Arcobacter isolates have been recovered (Atabay et al., 2006). However, arcobacters were not detected in any of the 210 cloacal swabs from broiler chickens from Malaysia (Amare et al., 2011).

Among the foods products of animal origin, the highest prevalence has been reported in chicken, followed by pork, beef and lamb meat. Reports indicate that arcobacters are frequently present in chicken meat (ranging from 23-100%) as compared to red meat (2-51.5%) (Kabeya et al., 2004; Lehner et al., 2005; Atabay et al., 2006; Scullion et al., 2006; Atanassova et al., 2008; Pentimalli et al., 2009). Based on these reports, chicken are regarded as significant reservoir of arcobacters and in particular chicken meat from retail market being an important source for spread of arcobacters (Gude et al., 2005; Pejchalova et al., 2008; Amare et al., 2011). Similar to the present report of 12% prevalence in variety of samples, recently Pejchalova et al. (2008) reported prevalence and diversity of Arcobacter spp. in a variety of samples viz. chicken, lamb, pork, beef and fish with in Czech Republic. An overall prevalence of 11.8% was observed with A. butzleri, A. cryaerophilus and A. skirrowii in 6.8, 5.1 and 0.2% of the samples, respectively. Similarly, 12.1% prevalence of arcobacters in various food, animal and water sources was observed in Turkey (Aydın et al., 2007). Recent studies indicated prevalence of Arcobacter in different food samples, types of meats and shellfish (Collado et al., 2009) with a very high prevalence in sea food (100%) and chicken (64.3%) followed by pork (53.0%), mussels (41.1%), duck (40.0%), turkey meat (33.3%) and beef (31.3%). In the present study, arcobacters were isolated from 10% of chicken meat, 5% of pork, 5% of beef samples and 1.67% of raw milk samples.

Not all the species of Arcobacter are considered as emerging and zoonotic in nature. Among Arcobacter spp. isolated, the prevalence of A. butzleri has been found most in meat samples, followed by A. cryaerophilus (Houf et al., 2003; Kabeya et al., 2004; Morita et al., 2004; Amare et al., 2011). The lower incidence of A. skirrowii may be either due to its low prevalence in meat or difficulty in isolation (Ho et al., 2006a; Snelling et al., 2006). There are reports indicating meats of chicken as an important source of A. butzleri (18.9%) and A. cryaerophilus (3.3%) in comparison to meat from pork and beef in Korea (Lee et al., 2010). Furthermore, Arcobacter spp. were found to be common contaminants of retail raw meats and raw milk in Northern Ireland. A. butzleri was the predominant species isolated from retail raw meats and the only species isolated from raw milk samples (Scullion et al., 2006). In contrast to above report, in the present study A. butzleri could be isolated only from the raw milk samples.

Analysis of human diarrheal stool samples indicated 2.0% prevalence rate of arcobacters during cultural methods and on PCR assy. Earlier reports have indicated that A. butzleri is the most common species associated with human disease while A. cryaerophilus isolated occasionally from humans with diarrhea and septicaemia; role of A. skirrowii in human disease is limited (Lerner et al., 1994; Engelberg et al., 2000; Gude et al., 2005; Ho et al., 2006a; Prouzet-Mauleon et al., 2006; Houf and Stephan, 2007; Samie et al., 2007). Atanassova et al. (2008) found A. butzleri having higher prevalence, followed by A. skirrowii and A. cryaerophilus in humans. The role of A. butzleri in Traveller's Diarrhea (TD) has been indicated (Jiang et al., 2010; Teague et al., 2010) recently; wherein only A. butzleri was found to be of zoonotic importance in human diarrheal cases. The presence of Arcobacter spp. in poultry may be of significance to human health and particularly chicken meat retailed in markets may render such meat a significant public health risk (Atabay et al., 2006; Atanassova et al., 2008; Amare et al., 2011).

Huge economic damage could be caused by Arcobacter species and sometimes infections could also become life threatening like Campylobacters.
The differences in recovery rates of Arcobacters from various sources could be due to geographic and seasonal variations, hygienic conditions and differences isolation methods (Collins et al., 1996; Atabay and Corry, 1998; Gonzalez et al., 2000; Atabay et al., 2003). Due to lack of standardized methods for isolation and cultural identification of Arcobacters, chances of isolation failures and misidentification are there (Houf et al., 2001b; Ho et al., 2006a; Snelling et al., 2006). Therefore, DNA-based assays would be more appropriate for rapid detection, confirmation and species level differentiation of arcobacters (Harmon and Wesley, 1997; Houf et al., 2000; Figueras et al., 2008). In the present study, PCR as the molecular method of bacterial detection was used. Earlier reports have indicated that PCR possess increased sensitivity, specificity and speed of identification of arcobacters in comparison to cultural methods (Harmon and Wesley, 1996; Gonzalez et al., 2000; Kabeya et al., 2003b; Prouzet-Mauleon et al., 2006; Gonzalez et al., 2007). Likewise in the present study, PCR screening of all the 400 samples after enrichment revealed 31 (7.75%) to be positive for Arcobacter in comparison to isolation of arcobacters from 27 samples by cultural methods. Few samples (04) failed to grow on selective solid media which may indicate difficulty in isolation of arcobacters. Also, results of the present study indicated direct application of PCR for the enriched samples to be better and less time consuming when compared to the cultural methods which is in conformity with report of Pentimalli et al. (2009).

From India, limited reports are available regarding isolation and detection of arcobacters from diarrheic human cases using either cultural methods or PCR assay (Kownhar et al., 2007; Jiang et al., 2010; Patyal et al., 2011; Bagalakote et al., 2013; Ramesh et al., 2014a). However, the present study employed both cultural and molecular tools of PCR as well as multiplex PCR simultaneously for species level identification of arcobacters. Furthermore, samples from different sources like animals and foods of animal origin were tested and compared. Analysis of PCR positive arcobacter isolates by multiplex-PCR assay revealed the presence of two arcobacter species (A. butzleri and A. skirrowii) in various samples. These results indicates the association of arcobacters in human and animal diseases and were in line with earlier reports by several other workers (Kabeya et al., 2003a; Atabay et al., 2006; Snelling et al., 2006; Aydin et al., 2007; Houf and Stephan, 2007; Patyal et al., 2011). With MP-PCR, out of a total of 27 PCR positive arcobacter isolates, 21 were A. butzleri and 6 were A. skirrowii. Multiplex PCR assay has been utilized for monitoring the presence of various Arcobacter spp. and identifying the pathogen at species level in different types of samples (Harmon and Wesley, 1997; Houf et al., 2000; Gude et al., 2005; Ho et al., 2006a; Scullion et al., 2006; Gonzalez et al., 2007; Pechelova et al., 2008; Pentimalli et al., 2009; Gonzalez and Ferrus, 2011; Amare et al., 2011). MP-PCR can also differentiate arcobacters from Campylobacter and Helicobacter, the other important enteropathogens in food products (Winters and Slavik, 2000; Neubaure and Hess, 2006). Thus the present study also suggests the effectiveness of the combined use of Arcobacter selective media with the genus based-PCR and MP-PCR for the isolation and detection of different Arcobacter spp. from various types of samples. By using a combination of PCR and culturing techniques, non-culturable as well as culturable forms of arcobacters could be thus detected.

Emergence of antibiotic resistant isolates or strains of many bacteria is a new challenge to the researchers worldwide (Triwari et al., 2013). In this context, an in vitro antibiotic sensitivity test was performed on PCR confirmed isolates of Arcobacter spp. in the present study. A total of 27 arcobacter isolates were subjected to in vitro antibiotic sensitivity test. It was found that most of the isolates were sensitive to azithromycin, gentamycin, nalidixic acid, kanamycin, streptomycin, ciprofloxacin and tetracycline; higher resistance was noted for cephalothin, novobiocin and
vancomycin and remarkable percentages of isolates were intermediately resistant against erythromycin and chloramphenicol. Previous reports have also indicated that *Arcobacter* isolates are resistant to many antibiotics like vancomycin, novobiocin and cephalothin (Houf *et al.*, 2001a; Atabay and Aydin, 2001). Thwaites and Frost (1999) reported that *Arcobacter* species showed susceptibility to aminoglycosides, including kanamycin and streptomycin. This relatively more resistant nature of arcobacters to different antibiotics coincides with studies of different workers indicating high resistance nature of arcobacters against trimethoprim (81.1%), sulphamethoxazole (87.2%) and members of the broad spectrum beta-lactams including cephalosporins (Kabeya *et al.*, 2004). Also, Houf *et al.* (2004) observed the acquisition of erythromycin and ciprofloxacin resistance which is of concern since these are frequently used as first-line drugs against human Campylobacteriaceae infections.

**CONCLUSION**

In conclusion, the present study reports the prevalence of *A. butzleri* and *A. skirrowii* species of arcobacters, known to possess zoonotic significance, in humans, animals and foods of animal origin in India based on cultural isolation, antibiogram, PCR and multiplex PCR detection. Although cultural methods detected the arcobacters, PCR proved to be more rapid and reliable with a detection of 7.75% of the test samples in comparison to cultural methods of 6.75%. The excretion of arcobacters in animal faeces could act as a potential source for carcass contamination during slaughter and this may pose threat to meat safety and human health, as these have been associated with enteritis in humans. Further large scale epidemiological studies involving molecular tests are required to determine prevalence and characterization of various *Arcobacter* spp. and the correlation between the human and animal infections. Development of effective preventive and control measures along with strengthening of diagnostic facilities and creating awareness regarding this important pathogen having public health concerns are suggested.

**REFERENCES**


