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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 6.75% (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 aerotolerent *campylobacters*

(Ellis *et al*., 1977, 1978; Lehner *et al*., 2005; Ho *et al*., 2006a). They are considered as emerging food-borne pathogens worldwide. The genus *Arcobacter,* belongs to Campylobacteraceae family (Vandamme *et al*., 1991). *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 lay, 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; Samie *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 (Fera *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*., 2006; Scullion *et al*., 2006). 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

			No. of samples detected positive for *Arcobacter* spp.			
			Conventional cultural methods		Standardized genus based PCR assay	
Type of samples	Source	No. of samples	No.	%	No.	%
Faecal samples						
Human diarrheal stool samples	Human hospital, Indian Veterinary Research Institute (IVRI), Izatnagar and different Private Hospitals, Izatnagar, Bareilly (Uttar Pardesh)	50	1	2	1	2
Poultry	Poultry farms, Central Avian Research Institute (CARI), Izatnagar and retail chicken market, Bareilly (Uttar Pardesh)	50	4	8	5	10
Pig	Pig farm, IVRI, Izatnagar, Bareilly (Uttar Pardesh)	50	6	12	6	12
Cattle	Dairy farm, IVRI, Izatnagar, Bareilly (Uttar Pardesh)	50	5	10	6	12
Food samples of animal origin						
Raw milk	Dairy farm, IVRI, Izatnagar, Bareilly (Uttar Pardesh)	60	1	1.67	1	1.67
Chicken	Slaughter house, CARI, Izatnagar and chicken retail shops, Bareilly (Uttar Pardesh)	60	6	10	7	11.67
Beef	Slaughter house, retail shops Bareilly (Uttar Pardesh)	40	2	5	3	7.5
Pork	Slaughter house, retail shops Bareilly (Uttar Pardesh)	40	2	5	2	5
Total		400	27	6.75	31	7.75

from retail shops and slaughter houses in and around Bareilly (Uttar Praedsh, 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 (16 mg L^{-1}), 5-fluorouracil (100 mg L^{-1}), Amphotericin 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% O_2, 10% CO_2 and 85% N_2) 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 1223 bp. Briefly, to 5 μL of 10X *Taq* buffer, 2.5 mM $MgCl_2$, 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 $MgCl_2$, 15 pmol of the primers (ARCO butZ, CRY-1, CRY-2 and SKIR), 5 μL heat lyses 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 H_2S 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

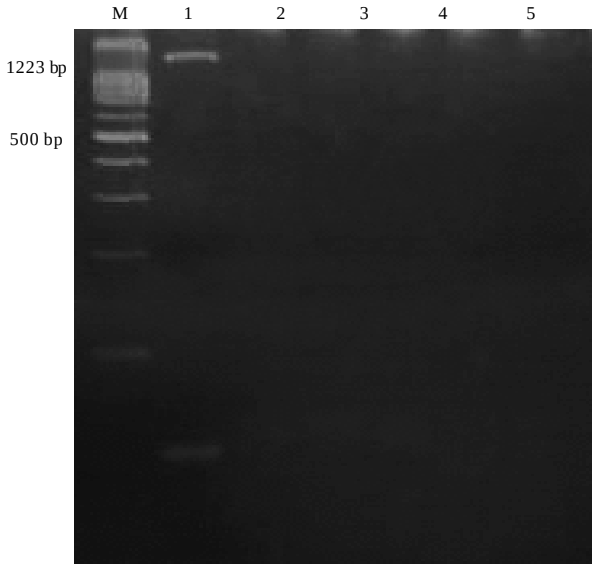

Fig. 1: Specificity of genus based PCR assay for *Arcobacter*. Lane M: 100 bp DNA ladder, Lane 1: *Arcobacter butzleri*, Lane 2: *C. Jejuni*, Lane 3: *C. coli*, Lane 4: *Salmonella typhimurium* and Lane 5: *Aeromonas* spp.

in detecting *A. butzleri* DNA level to the extent of 1 pg μL^{-1} concentration (Fig. 2). Appilcation 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

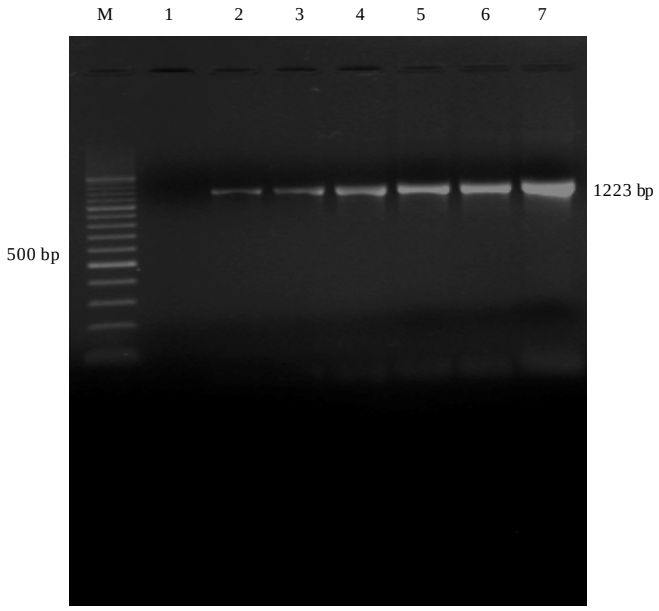

Fig. 2: Sensitivity of genus specific PCR assay for *Arcobacter*. Lane M: 100 bp DNA ladder, Lane 1: 0.1 pg μL^{-1}, Lane 2: 1 pg μL^{-1}, Lane 3: 10 pg μL^{-1}, Lane 4: 1×10^2 pg μL^{-1}, Lane 5: 1×10^3 pg μL^{-1}, Lane 6: 1×10^4 pg μL^{-1}, Lane 7: 5×10^4pg μL^{-1}

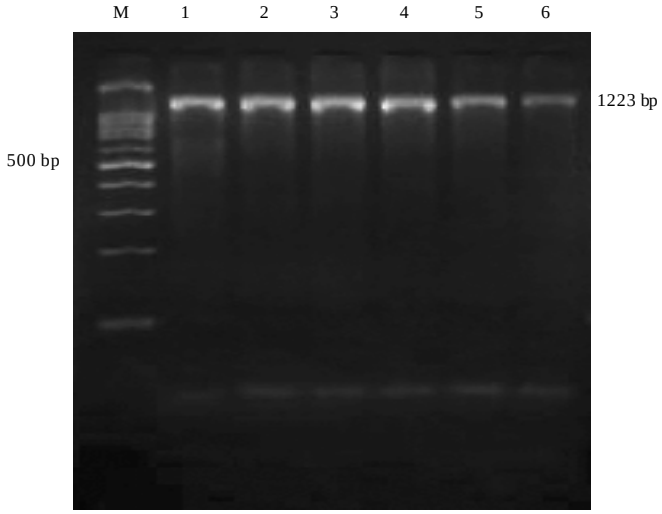

Fig. 3: Confirmation of *Arcobacter* isolates by genus based PCR. Lane M: 1 k bp DNA ladder, Lane 1: Positive control DNA of *Arcobacter butzleri* and Lane 2-6: Positive for genus *Arcobacter*

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 tetracycline (100, 100, 100,

Table 2: Detection of *Arcobacter* spp. by multiplex PCR in different samples

Culturally positive *Arcobacter* isolates recovered from different samples	*A. butzleri*	*A. skirrowii*	Total
Human diarrhoeic stool (1)	1	-	1
Poultry faeces (4)	4		4
Cattle faeces (5)	3	2	5
Pig faeces (6)	4	2	6
Raw milk (1)	1	-	1
Chicken (6)	4	2	6
Beef (2)	2	-	2
Pork (2)	2	-	2
Total	21	6	27

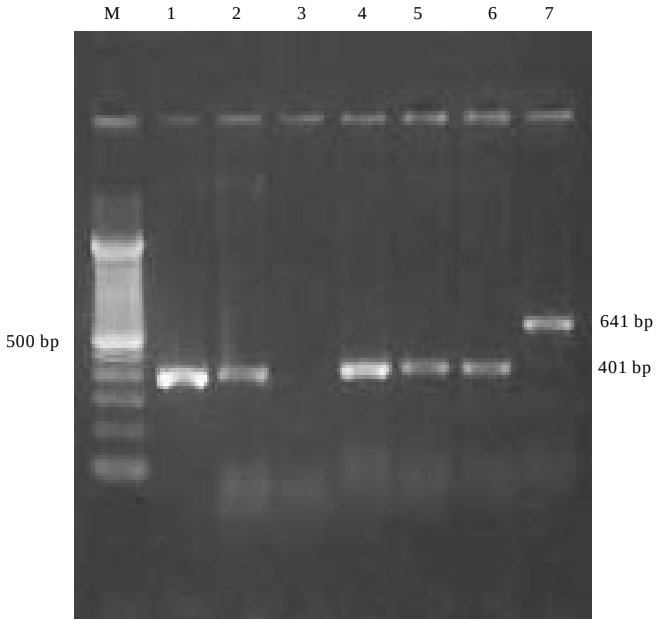

Fig. 4: Species level detection of *Arcobacter* isolates by multiplex PCR. Lane M: 1 k bp DNA ladder, Lane 1: Positive control DNA of *Arcobacter butzleri*, Lane 2, 4, 5 and 6: Positive for *Arcobacter butzleri,* Lane 3: Negative control and Lane 7: positive for *Arcobacter skirrowii*

Table 3: *In-vitro* antibiotic sensitivity profile of *Arcobacter* isolates

	Sensitive		Intermediate		Resistant	
Dose of antibiotics (µg)	No.	%	No.	%	No.	%
Azithromycin (30)	27	100.00	0	0.00	0	0.00
Ciprofloxacin (5)	25	92.59	2	7.40	0	0.00
Clindamycin (2)	22	81.48	5	18.51	0	0.00
Erythromycin (15)	2	7.40	25	92.59	0	0.00
Gentamicin (10)	27	100.00	0	0.00	0	0.00
Nalidixic acid (30)	27	100.00	0	0.00	0	0.00
Tetracycline (30)	24	88.88	3	11.11	0	0.00
Chloramphenicol (30)	0	0.00	24	88.88	2	11.11
Cephalothin (30)	0	0.00	0	0.00	27	100.00
Novobiocin (30)	0	0.00	2	7.40	25	92.59
Kanamycin (30)	26	96.29	1	3.70	0	0.00
Streptomycin (30)	26	96.29	1	3.70	0	0.00
Vancomycin (30)	0	0.00	2	7.40	25	92.59

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 intermediately 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 wells 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; Houf 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 3.6, 10 and 14.5% of the cattle and swine fecal samples and chicken cloacal swabs, respectively (Kabeya *et al*., 2003a). Also, Ongor *et al*. (2004) reported *Arcobacter* spp. in 9.5% of 200 rectal fecal 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 6.6, 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 (Aydin *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 assay. 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; Engberg *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; Ramees *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; Pejchalova *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 (Tiwari *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 aminoglycides, 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 (67.2%) 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 heath, 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.

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