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Detection of *Arcobacter* spp in poultry, pigs, their meat and environment samples by conventional and PCR assays

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ABSTRACT

Samples (300) comprising poultry and pig faeces, meat, poultry intestinal contents and environmental samples were investigated bacteriologically for the presence of *Arcobacter* spp. On the basis of morphology and biochemical tests, 34 (11.33%) of the isolates were identified as *Arcobacter*. The isolates grew at 28°C aerobically but failed to grow at 42°C. Arcobacters were differentiated from closely related campylobacters by their ability to grow in aerobic condition and negative for hippurate hydrolysis test. The genus specific amplification of 16S rRNA gene by PCR gave an amplification product of 1223 bp in all 34 presumptive *Arcobacter* isolates. The highest rate of *Arcobacter* isolation was from poultry meat samples (18%) followed by poultry environmental samples (16%), poultry intestinal contents (13.33%), poultry faecal (10%), pork (10%) and pig faecal (8%) and no arcobacters could be isolated from the pig environments. Multiplex PCR (m-PCR) targeting for 16S r RNA and 23S rRNA genes detected *A. butzleri* (12), *A. skirrowii* (6) and *A. cryaerophilus* (4). However, some of isolates showed mixed culture of both *A. butzleri* and *A. skkirrowii* (5), *A. skkirrowii* and *A. cryaerophilus* (4) and *A. butzleri* and *A. cryaerophilus* (3).

Arcobacter species are important food-borne pathogens detected in foods of animal origin (Amare *et al.* 2011). Consumption of raw and cross-contaminated undercooked meat constitute risk factors for human infection (Lehner *et al.* 2005). Water serves as an important vehicle for the transmission of these organisms and was a major factor in acquiring the diarrheal illness associated with *Arcobacter* spp. (Jacob *et al.* 1993). Some of the *Arcobacter* species were isolated from stool samples of patients with diarrhoea and occasionally in association with bacteremia (Ho *et al.* 2006). In animals, *Arcobacter* spp. is found associated with abortions, mastitis and gastrointestinal disorders and sometimes these organisms were recovered from asymptomatic animals (Vandamme *et al.* 1992). This study reports detection of *Arcobacter* species from different sources (animals faeces, animal meat and their environment) by conventional isolation and identification techniques, followed by PCR assays.

MATERIALS AND METHODS

Isolation and identification of Arcobacter *spp.:* Samples (300) comprising 100 faecal samples (50 poultry + 50 pig), 90 meat samples (50 poultry + 40 pig), 50 environmental samples (25 poultry + 25 pig) and 60 poultry samples (poultry intestinal contents), were collected and processed for isolation and characterization of *Arcobacter* spp. using standard conventional cultural isolation and identification methods. The faecal/intestinal swabs of poultry were inoculated into 10 ml *Arcobacter* broth supplemented with CAT (cefoperazone, amphotericin B, teicoplanin) with 5% defibrinated sheep blood for enrichment of *Arcobacter* spp. while the meat samples weighing 10 g were homogenized and suspended in 10 ml *Arcobacter* broth and the environmental samples were also processed in the same way. One ml of the homogenized suspension was inoculated into 9 ml of *Arcobacter* broth and the samples were incubated at 28°C for 48 h under microaerophilic (5% O_2, 10% CO_2 and 85% N_2) conditions. These samples which were enriched in broth were streaked onto the *Arcobacter* selective agar medium containing CAT antibiotic supplements and 5% defibrinated sheep blood and further incubated aerobically at 28°C for 48 h. The cultures were examined microscopically and subjected to recommended standard biochemical tests (Vandamme and De ley 1991, Harrab *et al.* 1998).

Biochemical tests: Biochemical tests including catalase, oxidase, nitrate reduction, hippurate hydrolysis test, indoxyl acetate hydrolysis, urease, H_2S production, growth at 25°C, 37°C and 42°C, and alpha hemolytic activity were

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performed for characterization of *Arcobacter* spp. isolates.

PCR for detection of genus Arcobacter: The procedure of Harmon and Wesley (1996) was used for the confirmation of suspected *Arcobacter* isolates.

The PCR was performed in thin walled PCR tubes in 50 µl of reaction volume containing 5 µl of 10X Taq buffer (with 20 mM $MgCl_2$, 100 mM KCl, 0.1M EDTA, pH 8.0), 2.5 µl of 2 mM deoxy-ribonucleotide triphosphate (dNTP), 2.5 U *Taq* polymerase, 5 µl template DNA and 50 pmol each primers set Arco-I and Arco-II designed from 16S-rRNA which yielded a product size of 1223 bp (Table 1). The positive control DNA of *A. butzleri* (LMG 10828T) was procured from the Belgium Bacterial Collection Centre (BCCM/LMG). The reaction was performed in a thermocycler having preheated lid (lid temp 105°C). The cycling condition comprised an initial denaturation at 94°C for 5 min, followed by 30 cycles each of denaturation at 94°C for 30 sec, primer annealing at 51°C for 30 sec, elongation at 72°C for 10 min and finally a single extension step at 72°C for 10 min was used in PCR. On completion of the reaction time, the amplified PCR products were electrophoresed on 1.5% agarose gel and analysed by using UV transilluminator. Specificity of standardized PCR assay and primers was checked with other bacterial DNA (*C. jejuni*, *C. coli*, *Salmonella*, *Aeromonas* and *E. coli*) extracted samples.

Multiplex PCR (m-PCR) for speciation: Multiplex polymerase chain reaction (m-PCR) was used for the identification and differentiation of different Arcobacters species in a single reaction. Recommended primers set (Table 1) for 16S r RNA and 23S rRNA genes were used for the identification of *Arcobacter* spp. (*A. butzleri, A. cryaerophilus and A. skirrowii*) (Houf *et al.* 2000). The DNA of *A. butzleri* (LMG 10828T) was used as positive control in the present study. The reaction mixture of 50 µl was composed of 5 µl of 10X Taq buffer (with 20 mM $MgCl_2$, 100 mM KCl, 0.1M EDTA, pH 8.0), 2.5 µl of 2 mM deoxy-ribonucleotide triphosphate (dNTP), 2.5 U *Taq* polymerase, 5 µl template DNA and 50 pmol of each primer ARCO, BUTZ, CRY–1, CRY-2 and 25 pmol of SKIR primer. The m-PCR involved an initial denaturation at 94°C for 5 min, followed by 30 cycles each of denaturation (94°C for 30 sec), primer annealing (51°C for 30 sec) and extension (72°C for 1 min), and final extension was done at 72°C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel and analysed using UV transilluminator.

RESULTS AND DISCUSSION

Depending upon colonial morphology, motility, Gram's staining and biochemical characteristics, 34 (11.33%) of the isolates were identified as *Arcobacter*. All the presumptive *Arcobacter* isolates (34) were found Gram-negative, spirally curved rod or short 'S' shape organisms, showed cork screw darting type of motility. Biochemically, the organisms were positive to oxidase, catalase, indoxyl acetate hydrolysis and nitrate reduction test and negative to urease, hippurate hydrolysis test and H_2S production in triple sugar iron agar (TSI) medium, along with sensitivity to nalidixic acid and resistance to cephalothin. All the isolates showed growth at 28°C aerobically but no growth at 42°C. Arcobacters were differentiated from campylobacters particularly by their ability to grow in aerobic condition and negative for hippurate hydrolysis test.

Genus specific detection by PCR: All 34 isolates yielded an amplification product of 1223 bp corresponding for genus *Arcobacter*.

Multiplex PCR (m-PCR): All 34 *Arcobacter* which were positive to genus Arcobacter based-PCR assay were further subjected for multiplex-PCR assay used primers (Table 1)

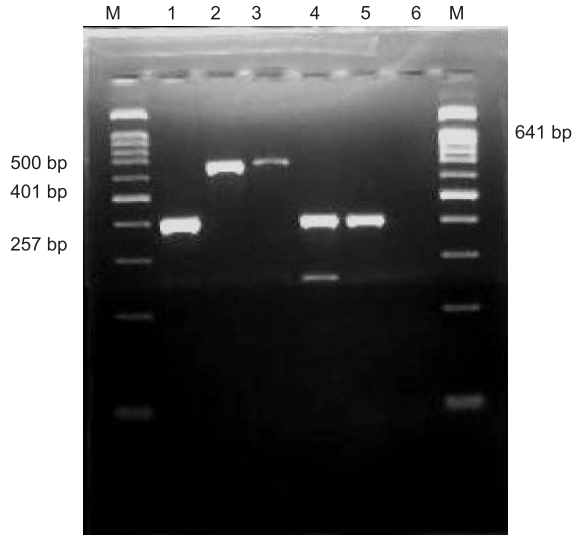

Fig. 1. Multiplex PCR based confirmation of *Arcobacter* spp. Lane M: 100 bp DNA ladder; lane 1, positive control DNA of *Arcobacter butzleri* (401 bp); lane 2 and 3, positive for *Arcobacter skirrowii* (641bp); lane 4, positive for *Arcobacter butzleri* and *Arcobacter cryaerophilus* (257 bp); lane 5, positive for *Arcobacter butzleri.*

Table 1. Primers for the PCR and m-PCR assays

Primar	Genes	Position	Nucleotide sequence (5'–3')	Reference
Arco I	16S rRNA	224-244	AGAGATTAGCCTGTATTGTAT	Harmon *et al.* (1996)
Arco II	16S rRNA	1426–1446	TAGCATCCCCGCTTCGAATGA	Harmon *et al.* (1996)
BUTZ	16S rRNA	959–983	CCTGGACTTGACATAGTAAGAATAG	Houf *et al.* (2000)
ARCO	16S rRNA	1357–1338	CGTATTCACCGTAGCATAGC	Houf *et al.* (2000)
SKIR	16S rRNA	705–723	GGCGATTTACTGGAACAC	Houf *et al.* (2000)
CRY 1	23S rRNA	105–124	TGCTGGAGCGGATAGAAGTA	Houf *et al.* (2000)
CRY 2	23S rRNA	359–340	AACAACCTACGTCCTTCGAC	Houf *et al.* (2000)

for species differentiation. Of 34 *Arcobacters*, 12 isolates showed positive result for *A. butzleri,* 6 for *A. skirrowii* and 4 were positive for *A. cryaerophilus*. However, 12 isolates were mixed culture of both *A. butzleri* and *A. skkirrowii* (5), *A. skkirrowii* and *A. cryaerophilus* (4) and *A. butzleri* and *A. cryaerophilus* (3).

The highest rate of *Arcobacter* isolation was obtained from poultry meat samples (18%) followed by poultry environmental samples (16%), poultry intestinal contents (13.33%), poultry faecal (10%), pork (10%) and pig faecal (8%) and no arcobacters could be isolated from the pig environments. *A. butzleri* is reported as the fourth most common *Campylobacter* like organism isolated from stool samples of human patients after *C. jejuni, C. coli* and *C. upsaliensis* in Belgium and France (Prouzet-Mauleon *et al*. 2006). Jiang *et al*. (2010) reported the presence of *Arcobacter* spp. in 201 U.S. and European travelers with acute diarrhea acquired in Mexico, Guatemala and India and of all diarrheal cases, *A. butzleri* was predominantly isolated from 8% cases. The association of *A. butzleri* and *A. cryaerophilus* with enteritis in humans and their recovery from chickens, sea food and pigs indicated a possible association of *Arcobacter* spp. with pork, sea food and poultry products (Collado *et al*. 2009). Mohan (2008) observed the prevalence of *Arcobacter* spp. in raw milk, chicken meat, beef and pork samples to be 1.66, 10, 5 and 5%, respectively, and out of all isolates, 81.81% isolates were *A. butzleri* and 18.18% were *A. skirrowii.* In the same study, the prevalence of *Arcobacter* spp. from human diarrheal stool samples, cattle, poultry and porcine fecal samples was 2, 10, 8 and 12%, respectively, and of all isolates, 75% isolates were positive for *A. butzleri* and 25% for *A. skirrowii.* The overall prevalence of *Arcobacter* in Bareilly (Uttar Pradesh) was reported to be 10.50% (Patyal *et al.* 2011). The isolation rate was higher in poultry meat (18%) than pig meat (10%). Kabeya *et al.* (2004) reported highest prevalence of arcobacter in chicken (23%) followed by pork (7%) and lowest in beef (2.2%). An even higher prevalence was also observed by Rivas *et al.* (2004) where 73% of chicken, 29% of pork and 22% of beef samples were positive for arcobacters in Australia. Similarly, to meat samples, faeces of the poultry (10%) also showed higher isolation rate than pig faeces (8%). Their findings were similar to the observations of Kabeya *et al.* (2003) who reported that 10% of the swine faeces samples and 14.5% of chicken cloacal swabs were positive for *Arcobacter* spp. *Arcobacter* was also isolated from 43.5% of porcine faecal samples (Van Driessche *et al*. 2003). These studies suggested pig and poultry may be reservoirs of *Arcobacter* infection in human beings. The difference between rates of isolation in the various studies may be attributed due to variations in sampling, the isolation procedures and managemental practices used in different countries. In our study, the isolation rate in the intestinal contents was 13.33%. Our finding was consistent with the observation of Ho *et al.* (2008) where the *Arcobacter* spp. were detected in the gut system of chickens, ranging from 20 to 85% in hens and 3.3 to 51% in broilers. The isolation rate of *Arcobacter* in the poultry environment was 16%, while in pig environmental samples none was found to be positive for *Arcobacter*, so presence of the *Arcobacter* in poultry environmental may be suggestive of higher prevalence of the organism in this species. Application of standardized m-PCR assay in this study, detected 3 different species of *Arcobacter* genus, viz. *A. butzleri* (12)*, A. skirrowii* (6) and *A. cryaerophilus* (4). whereas 12 showed mix culture of *A. butzleri* and *A. skirrowii*, *A. butzleri* and *A. cryaerophilus* and *A. skirrowii* and *A. cryaerophilus*. Houf *et al.* (2000) has also reported the mixed infection of *A. btuzleri* and *A. cryaerophilus*. This study concluded that *Arcobacter* spp., were present in faecal samples of animals (pig and poultry) and meat samples of animal (pork and chicken) which may contaminate environment and human food chain as well.

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REFERENCES

Amare L B, Saleha A A, Zunita Z, Jalila A and Hassan L. 2011. Prevalence of *Arcobacter* spp. on chicken meat at retail markets and in farm chickens in Selangor, Malaysia. *Food Control* **22**: 732–36.

Collado L, Cleenwerck I, Trappen S V, Vos P D and Figueras M J. 2009. *Arcobacter mytili sp*. nov. isolated from Mussels in Spain. *International Journal of Systematic and Evolutionary Microbiology* **59**: 1391–96.

Harrab B, Schwarz S and Wenzel S. 1998. Identification and characterization of *Arcobacter* isolates from broilers by biochemical tests, antimicrobial resistance patterns and plasmid analysis. *Journal of Veterinary Medicine* **45**: 87–94.

Harmon K M and Wesley I V. 1996. Identification of *Arcobacter* isolates by PCR. *Letters Applied Microbiology* 2**3**: 241–44.

Ho H T, Lipman L J and Gaastra W. 2006. *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent. *Veterinary Microbiology* **115**: 1–13.

Ho T K H, Lipman L J A and Gaastra W. 2008. The introduction of *Arcobacter* spp. poultry slaughterhouses. *International Journal of Food Microbioogy* **125**: 223–29.

Houf K, De Zutter L, Van Hoof J and Vandamme P. 2003. Molecular characterization of *Arcobacter* isolates collected in a poultry slaughter house. *Journal of Food Protection* **66**: 364–69.

Houf K, On S L, Coenye T, Mast J, Van Hoof J, Vandamme P 2005. *Arcobacter cibarius*sp. nov., isolated from broiler carcasses. *International Journal of Systematic Evolutionary Microbiology* **55(2)**: 713–17.

Houf K, On S L, Coenye T, Debruyne L, De Smet and S, Vandamme P. 2009. *Arcobacter thereius* sp. nov., isolated from pigs and ducks. *International Journal of Systematic Evolutionary Microbiology* **59**: 2599–604.

Jacob J, Loir H and Feuerpfeil I. 1993. Isolation of *Arcobacter butzleri* from drinking water reservoir in Eastern Germany. *Zentralblatt-fuer-Hygiene* 1**93**: 557–62.

Jiang Z, DuPont, H L, Brown E L, Nandy R K, Ramamurthy T, Sinha A, Ghosh S, Guin S, Gurleen K, Rodrigues S, Chen J J,

McKenzie R and Steffen R. 2010. Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and *Arcobacter* species. *Journal of Clinical Microbiology* **48(4)**: 1417–19.

Kabeya H, Maruyama S, Morita Y, Kubo M, Yamamoto K, Arai S, Izumi T, Kobayashi Y, Katsube Yand Mikami T. 2003. Distribution of *Arcobacter* species among livestock in Japan. *Veterinary Microbiology* **93**: **1**53–58.

Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y. and Mikami, T. 2004. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *International Journal of Food Microbiology* **90**: 303–08.

Lehner A, Tasara T, Stephan R. 2005. Relevant aspects of *Arcobacter spp*. as potential food-borne pathogens. *International Journal of Food Microbiology* **102**: **1**27–35.

Mohan H V. 2008. 'Detection of *Arcobacter* spp. in man, animals and foods of animal origin by using cultural and PCR techniques.' M.V.Sc Thesis, Deemed University, IVRI, Izatnagar.

Patyal A, Rathore R S, Mohan H V, Dhama K and Kumar A. 2011. Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin including sea food from India. *Transboundary and Emerging Diseases* **58 (5)**: 402–10.

Prouzet-Mauleon V, Labadi L, Bouges N, Menard A and Megraud F. 2006. *Arcobacter butzleri*: underestimated enteropathogen. *Emerging Infectious Diseases* **12**: 307–09.

Van Driessche E, Houf K, Vangroen weghe F, De Zutter L and Van Hoof J. 2003. Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. *Veterinary Microbiology*. **105(2)**: **1**49–54.

Vandamme P and De Ley J.1991. Proposal for a new family, *Campylobacteraceae*. *International Journal of Systematic Bacteriology* **41**: 451–55.

Vandamme P, Vancanneyt M, Pot B, Mels L, Hoste B, Dewettinck D, Vlaes L, van den Borre C, Higgins R and Hommez J. 1992. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleria* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *International Journal of Systemtic Bacteriology* **42(3)**: 344–56.