Real-Time PCR Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in Chicken Meat Samples

**Thadiyam Puram Ramees¹*, Ramswaroop Singh Rathore¹,
Prashanth Suresh Bagalkot¹, G.V.P.P.S. Ravi Kumar², Hosakote Venkatappa
Mohan¹, R. Anoopraj³, Ashok Kumar¹ and Kuldeep Dhama³**

¹Division of Veterinary Public Health, Indian Veterinary Research Institute,
Izatnagar, Bareilly (U.P.) – 243122, India.
²Animal Biotechnology Division, Indian Veterinary Research Institute,
Izatnagar, Bareilly (U.P.) – 243122, India.
³Division of Pathology, Indian Veterinary Research Institute,
Izatnagar, Bareilly (U.P.) – 243122, India.

Arcobacter is an important and emerging food borne pathogen worldwide, which causes gastroenteritis and occasional septicaemia in human beings. The conventional cultural methods and traditional diagnostic tools are laborious and take long time to detect *Arcobacter* spp., and misidentification warrants comparing with molecular method(s). Therefore, the present study was designed for optimising real time - polymerase chain reaction (PCR) using SYBR green PCR master mix and published primers of multiplex PCR, for detection of two *Arcobacter* spp. (*Arcobacter butzleri* and *Arcobacter cryaerophilus*) and apply it for screening of chicken meat samples for Arcobacters. Real time PCR assay was used for the detection of *Arcobacter* from the enriched broth and out of a total of 50 chicken meat samples collected from different retail meat shops of Bareilly region, Uttar Pradesh, India, 28 (56%) samples were found positive for *Arcobacter* spp. This is the first report from India regarding optimization and applicability of real-time PCR for detection of Arcobacters in meat samples. Reports regarding detection and prevalence of Arcobacters from the country are very few, and in this scenario the wide applicability of the molecular tool of real-time PCR is suggested for knowing the real prevalence and magnitude of *Arcobacter* infection in a variety of clinical samples of animals and humans as well as various sources of foods.

Key words: *Arcobacter*, Chicken meat, Real-time PCR, SYBR green.

Arcobacter is an important food and water borne pathogen associated with enteritis in human beings and abortion, mastitis and diarrhoea in animals[1,2,3,4]. Arcobacters falls under the family *Campylobacteraceae* and can be recovered from a variety of foods of animal origin (beef, pork, milk) and animal/poultry meat[5,6,7]. It is also associated with bovine abortion and can be isolated from the preputial washing of bulls[8,9]. The most prevalent species is *Arcobacter butzleri* compared to *Arcobacter cryaerophilus* in healthy animals[10,11]. Real time -polymerase chain reaction (PCR) assay is a more sensitive and rapid method for detection of *Arcobacter* spp.[12]. A real-time fluorescence resonance energy transfer PCR method has been validated for *Arcobacter* spp.[13]. This has been shown to be specific, reproducible, and accurate method with a detection level of 5.6×10 CFU/mL. A genus specific SYBR Green real-time polymerase chain reaction (PCR) assay was also developed for *Arcobacter* detection in food and wastewater samples by Gonzalez et al.[14], with high sensitivity

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* To whom all correspondence should be addressed.
Tel.: +91-9458546916(M);
E-mail: rameesvet@gmail.com

and specificity for detection of *Arcobacter*. This SYBR green assay showed enhanced detection levels than conventional PCR. Further, *A. butzleri* from stool samples was detected by use of real-time multiplex PCR with high sensitivity and specificity[15]. Therefore, the present study was aimed to optimize real time PCR and apply it for detection of two important *Arcobacter* spp. (*Arcobacter butzleri* and *Arcobacter cryaerophilus*) in chicken meat samples.

MATERIALS AND METHODS

Sample collection, processing and DNA preparation

A total number of 50 chicken meat samples were collected from retail meat shops from in and around Bareilly region, Uttar Pradesh, India. Ten grams of chicken meat samples were aseptically minced with scissors and suspended in 90 ml of phosphate buffer solution (PBS, pH 7.0). The mixtures were homogenized with stomacher for 1 min at 200 revolutions per minute (rpm). One ml of the suspension was inoculated into 10 ml of CAT broth and incubated at 30°C under microaerophilic condition for 48 hrs for enrichment. The whole cell DNA was extracted from all the 50 enriched samples by heat lysis (snap chill) method.

Optimization of Real-time PCR assay with SYBR Green Master mix

The real-time PCR assay using primer sets as used earlier for multiplex PCR testing [ABUTZF (forward) and AGENR (reverse)[12] for *Arcobacter butzleri,* and CRY 1 (forward) and CRY 2 (reverse)[16] for *Arcobacter cryaerophilus*] was standardized as per method of Gonzalez et al.[14] with slight modifications during optimization. Real-time PCR assay was performed with quantifast SYBRGreen Master Mix (QIAGEN) and Mx3000P spectrofluremetric thermal cycler operated by MxProTM PCR software. Real time PCR reaction was run in triplicate, each containing 0.60 µl each of forward and reverse primers (10 pMol/ µl), 3.75 µl template DNA and 12.5 µl SYBRGreen QPCR Master Mix and nuclease-free PCR grade water to make upto 25 µl reaction mixture. The PCR conditions included initial denaturation at 95°C for 10 min (segment I), denaturation at 95°C for 30 s, annealing at 58°C for 30 sec and extension at 72°C for 30 sec for 40 cycles (segment II). Fluorescence was recorded at the end point of each cycle. Dissociation (melting) curve analysis consisted of 95°C for 1 min, followed by 58°C for 30 s with gradual increment from 58°C to 95°C @ 2 degree per min and 95°C for 30 sec (segment III).

Specificity of the real time PCR assay

Specificity of standardized PCR real time assay and primers was checked with other bacterial cultures (*Campylobacter jejuni*, *Campylobacter coli*, *Salmonella spp.* and *Escherichia coli*) by preparing templates using whole cell heat lysates and subjecting to real time PCR assay together with known positive and negative controls.
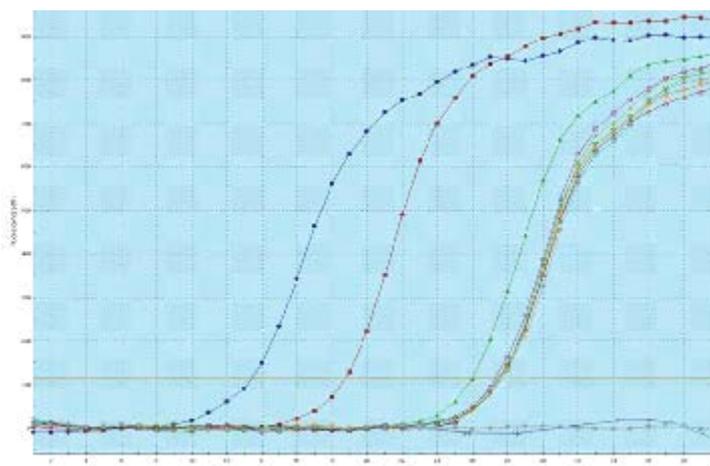

Fig. 1. Real time PCR amplification plot for four *A. butzleri*, four *A. cryaerophilus* and two negative controls.

RESULTS AND DISCUSSION

Real time PCR assay was used for the detection of *Arcobacter* from the enriched broth. Out of a total of 50 chicken meat samples tested, 28 (56%) samples were positive with 11 showed *A. butzleri* only, 8 showed *A. cryaerophilus* only and 9 showed presence of both *A. butzleri* and *A. cryaerophilus*. Real time PCR amplification plots and dissociation curves are shown in Figure 1 and 2. The real time PCR was found to be highly specific for *Arcobacter* spp. as no amplification was observed for other enteropathogens as shown in figure 3.

Arcobacter spp. are considered as important and emerging food borne and water borne pathogens worldwide, causing human and animal illness. Arcobacters have been isolated from different foods of animal origin (chicken meat, pork, beef, mutton, milk), and clinical samples (faecal samples of animals and human stools) with varying prevalence rates[11,17,18]. Compared to conventional cultural and traditional diagnostic methods the molecular tools of PCR and its allied versions (multiplex PCR, real-time PCR, quantitative PCR) have been reported to be highly useful for detecting and knowing the prevalence rates of food borne and enteric pathogens like Arcobacters[3,7,11,18].
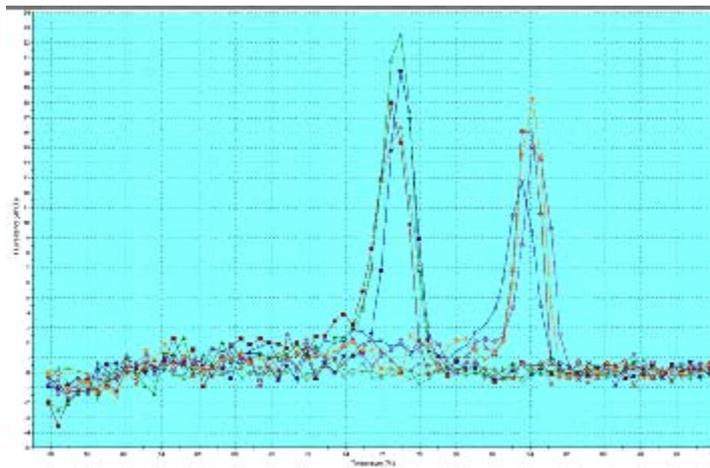

Fig. 2. Real time PCR dissociation curve for four *A. butzleri*, four *A. cryaerophilus* and two negative controls
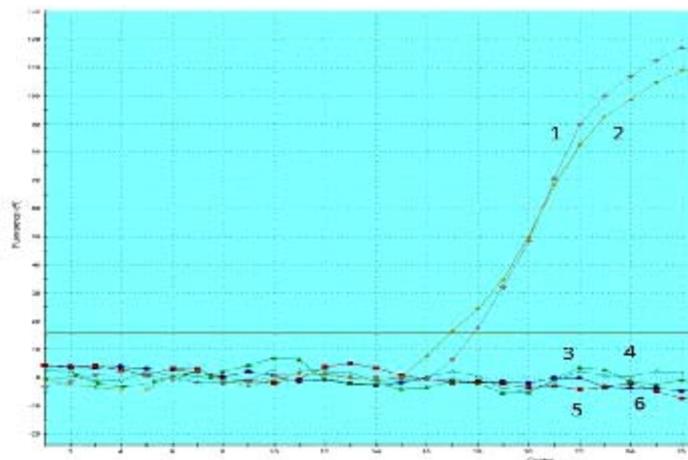

Fig. 3. Real time PCR specificity testing for *Arcobacter* spp.
1: *A. butzleri* ; 2 : *A. cryaerophilus* ; 3: *Salmonella* spp.; 4 : *Escherichia coli* ; 5: *Campylobacter jejuni* ; 6: *Campylobacter coli*

A 80.5% (29/36) detection rate was reported from meat samples by genus specific SYBR green real time PCR assay[14]. In earlier studies from India, in chicken meat, 12%[3], 39.42%[19] and 32.45 %[20] prevalence rates were reported by genus specific multplex-PCR, indicating high sensitivity of real time PCR in the present study. More recently, Rahimi [21] reported 31 % prevalence from meat samples from Iran by multiplex-PCR. Prevalence rates of 23%, 32% and 39.2% for *Arcobacter* spp. in retail chicken meat were reported in Japan[6], Nigeria[22] and Malaysia[7] respectively. A global prevalence of 32% was reported from different meat samples and shellfish by multiplex PCR[23]. In the current scenario of emerging antibiotic resistant bacterial strains (*C. jejuni*, *E. coli*, *L. monocytogenes* and *Arcobacter spp.*) and food-borne pathogens worldwide the significance of their rapid detection and studying prevalence arises, which would help in designing suitable countering strategies against these pathogens having high public health concerns and protect the health of humans and their companion animals[24,25,26]. In this context, the real time PCR assay for detection of *Arcobacter* spp. was found to be highly sensitivity and specificity as reported by earlier workers[12, 14].

CONCLUSION

The present study reports the optimization and applicability of molecular tool of real time PCR, having higher sensitivity and specificity, for detection of Arcobacters in chicken meat samples with overall prevalence rate of 56%. Reports being very scarce from India regarding detection and prevalence of Arcobacters, the wide applicability of the of real-time PCR standardized is suggested for revealing the prevalence and epidemiological picture of this important and emerging pathogen in a variety of clinical samples of animals and human origin and various food sources, which would help devising appropriate prevention and control measures for countering it.

REFERENCES

1.	Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R., de Ley, J. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* genera. *Int. J. Syst. Bacteriol.*, 1991; **41**: 88-103.

2.	Engberg, J., On, S.L.W., Harrington, C.S., Gerner-Smidt, P. Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in Human Fecal Samples as Estimated by a Reevaluation of Isolation Methods for Campylobacters. *J. Clin. Microbiol.*, 2000; **38**(1): 286–291.

3.	Patyal, A., Rathore, R.S., Mohan, H.V., Dhama, K., Kumar, A. Prevalence of *Arcobacter* spp. in Humans, Animals and Foods of Animal Origin Including Sea Food from India. *Transbound Emerg Dis.*, 2011; **58**: 402–410.

4.	Merga, Y.J., Williams, N.J., Miller, W.G, Andrew J.H., Leatherbarrow., Bennett, M., Hall, N., Ashelford, K.E., Winstanley, C. Exploring the Diversity of *Arcobacter butzleri* from Cattle in the UK Using MLST and Whole Genome Sequencing. *PLOS ONE.*, 2013; **8**(2): e55240.

5.	Houf, K., De Zutter, L., Van Hoof, J., Vandamme, P. Occurrence and distribution of *Arcobacter* species in poultry processing. *J. Food Protect.*, 2002; **65**: 1233-1239.

6.	Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y. and Mikami, T. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int. J. Food Microbiol.*, 2004; **90**: 303-308.

7.	Amare, L.B., Saleha A.A., Zunita, Z., Jalila, A., Hassan, L. Prevalence of *Arcobacter* spp. on chicken meat at retail markets and in farm chickens in Selangor, Malaysia. *Food Control.*, 2011; **22**: 732–736.

8.	Logan, E. F., S. D. Neill., D. P. Mackie. Mastitis in dairy cows associated with an aerotolerant campylobacter. *Vet. Rec.*, 1982; **110**: 229–230.

9.	Collado, L., Figueras M.J. Taxonomy, Epidemiology, and Clinical Relevance of the Genus *Arcobacter*. *Clin. Microbiol. Rev*., 2011; **24**(1): 174–192

10.	Ongor, H., Cetinkaya, B., Acik, M.N., Atabay, H.I., Investigation of Arcobacters in meat and faecal samples of clinically healthy cattle in Turkey. *Lett. Appl. Microbiol.*, 2004; **38**: 339–344.

11.	Ho, H.T, Lipman, L.J., Gaastra W. *Arcobacter*, what is known about a potential foodborne zoonotic agent!. *Vet. Microbiol.*, 2006; **115**: 1–13.

12.	Brightwell, G., Mowat, E., Clemens, R., Boerema, J., Pulford, D. J., On, S. L. Development of a multiplex and real time PCR

assay for the specific detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus*. *J. Microbiol. Methods.*, 2007; **68**: 318–325.

13. Abdelbaqi, K., Buissonniere, A., Prouzet-Mauleon, V., Gresser, J., Wesley, I., Megraud, F., Menard, A. Development of a real time fluorescence resonance energy transfer PCR to detect *Arcobacter* species. *J. Clin. Microbiol.,* 2007; **45**: 3015–3021.

14. Gonzalez, A., Suski, J., Ferrus, M.A.. Rapid and Accurate Detection of *Arcobacter* contamination in commercial chicken products and wastewater samples by real-time polymerase chain reaction. *Foodborne Pathog Dis.,* 2010; **7**(3): 327-338.

15. De Boer, R.F., Ott, A, Guren, P., Van Zanten, E., Van Belkum, A., Kooistra-Smid, A.M. Detection of *Campylobacter* species and *Arcobacter butzleri* in stool samples by use of real-time multiplex PCR. *J. Clin. Microbiol.*, 2013; **51**(1): 253-259.

16. Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J., Vandamme, P. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Immunol. Med. Microbiol.,* 2000; **49**(3): 337-45.

17. Vandenberg, O., Dediste, A., Houf K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N, Zissis, G., Butzler, J.P., Vandamme, P. *Arcobacter* species in humans. *Emerg. Inf. Dis.*, 2004; **10**: 1863-1867.

18. Wang, X., Seo, D.J., Lee, M.H., Choi, C. Comparison of conventional PCR, multiplex PCR, and loop-mediated isothermal amplification for rapid detection of *Arcobacter* species. *J. Clin. Microbiol*. 2013; doi:10.1128/JCM.02883-13.

19. Bagalakote, P.S., Rathore, R.S,. Ramees, T.P., Mohan, H.V., Sumankumar, M., Upadhyaya P.,

Bhilegaonkar K.N., Kumar, A. Study on prevalence of *Arcobacter* spp. in human and foods of animal origin. *J. Vet. Pub. Hlth.*, 2013; **11**(2): 105-109

20. Ramees, T.P., R.S. Rathore, P.S. Bagalkot, H.V. Mohan, A. Kumar., K. Dhama Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in clinical samples of humans and foods of animal origin by cultural and multiplex PCR based methods. *Asian J. Anim. Vet. Adv.* 2014; **9**(4): 243-252.

21. Rahimi, E. Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. *Br. Poult. Sci.*, 2014; DOI: 10.1080/00071668.2013.878783

22. Adesiji Yemisi, O., Alli Oyebode, T., Adekanle Margaret, A., Jolayemi Justina, B. Prevalence of *Arcobacter*, *Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone J Biomed Res.*, 2011; **3**: 8-12.

23. Collado, L., Guarro, J., Figueras, M. Prevalence of *Arcobacter* in meat and shellfish. *J. Food Protect.*, 2009: **72**: 1102-1106.

24. Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A., Tiwari, R., Kapoor, S. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pak. J. Biol. Sci.*, 2013a; **16** (20): 1076-1085.

25. Dhama, K., Chakraborty, S., Kapoor, S., Tiwari, R., Kumar, A., Deb, R., Rajagunalan, S., Singh, R., Vora, K., Natesan, S. One world, one health - veterinary perspectives. *Adv Anim Vet Sci.*, 2013b; **1**(1): 5-13.

26. Tiwari, R., Chakraborty, S., Dhama, K., Rajagunalan, S., Singh, S.V.. Antibiotic resistance - an emerging health problem: causes, worries, challenges and solutions – a review. *Int. J. Curr. Res.,* 2013; **5**(07): 1880-1892.