International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (5), pp. 001-007, May, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of sensitivity, specificity and species discriminatory power of four culture-based isolation methods of *Arcobacter* spp

Shah A. H.[1], Saleha A. A.[1*], Murugaiyah M.[2] and Zunita Zakaria[1]

[1]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

[2]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Accepted 13 March, 2019

A total of 142 samples including beef (n = 52), fresh milk (n = 45) and rectal swabs of cattle (n = 45) were collected from various wet markets (beef) and dairy cattle farms (milk and rectal swabs) in the vicinity of Selangor, Malaysia. All samples were examined for the presence of *Arcobacter* species using four different isolation methods. The organisms were first identified on the basis of phenotypic tests and later the suspected isolates were confirmed using multiplex PCR (mPCR). Method I (MI) and Method II (MII) detected *Arcobacter* in 43.7 and 75%, respectively from beef, but were unable to detect microbe from milk and rectal swabs. On the other hand, Method III (MIII) detected *Arcobacter* in 100, 60 and 40% of beef, milk and bovine rectal swab samples respectively; Method IV (MIV) detected 93.7, 60 and 40% in beef, milk and cattle rectal swabs respectively. The discriminatory power among the isolation methods for *Arcobacter* species was evaluated and MIII was found to be the best as it identified *A. butzleri* (72.7%); *A. cryaerophilus* (22.7%) and *A. skirrowii* (4.5%) while *A. butzleri* (61.1%), *A. cryaerophilus* (22.7%) and *A. skirrowii* (9%) were isolated from MIV.

Key words: *Arcobacter*; isolation methods, mPCR, beef, milk, cattle

INTRODUCTION

The members of the genus *Arcobacter* are motile, Gram negative, non-spore forming, curved, or sometimes appearing as spiral rods (Vandamme et al., 1991). *Arcobacter* was initially known as aerotolerant *Campylobacter* (Neill et al., 1979) and was isolated for the first time from aborted bovine fetuses in 1977 (Ellis et al., 1977), and porcine aborted fetuses the following year (Ellis et al., 1978).

The public health significance of the genus *Arcobacter*, in recent years, has become increasingly important because of emergence of more new species and their zoonotic potential. Until 2006 the genus *Arcobacter* consisted of only five species and currently seven new species have emerged (Shah et al., 2011; Figueras et al., 2010). All these 12 species have been isolated from various animal origin food products including beef (Aydin et al., 2007; De Smet et al., 2010), rabbit meat (Collado et al., 2009b), duck meat (Houf et al., 2009) and milk (Pianta et al., 2007; Sculllion et al., 2006); sea food (Collado et al., 2009a; Figueras et al., 2010), pork (Shah et al., 2011; Kabeya et al., 2004); animals such as cattle, horse, sheep, pigs, monkeys, raccoons, poultry (Ho et al., 2006; Van Driessche et al., 2005) as well as different types of water such as river, canals and drinking water (Ertas et al., 2010; Jacob et al., 1998; Musamanno et al., 1997).

Arcobacter species are associated with diseases such as mastitis and abortions in animals (Ho et al., 2006; On et al., 2002; Skirrow, 1994) and enteritis and septicemia in humans (Engberg et al., 2000; Prouet-Mauleon et al., 2006). Its pathogenicity is in dispute in the absence of fulfilling Koch's postulates. It has also been isolated from clinically healthy humans (Houf and Stephan, 2007).

Arcobacter spp. have been recognized as a potential food and water-borne pathogens (Gonzalez et al., 2007; Ho et al., 2006; Shah et al., 2011) however, standardized

*Corresponding author. E-mail: saleha@vet.upm.edu.my.

detection methods have yet to be established. In this perspective, a series of methods have been described employing various broths and supplements (Atabay and Corry, 1998; De Boer et al., 1996) and several studies comparing different culture based methods have been published (Johnson and Murano, 1999; Ohlendorf and Murano, 2002; Scullion et al., 2004). However, there is no recommended "standard method". Moreover, routine identification by means of phenotypic tests often gives erratic results due to the close phylogenetic relatedness of *Arcobacter* to *Campylobacter.* Absence of a reliable identification scheme may lead to significant under-estimation of the actual prevalence of *Arcobacter* (Harrab et al., 1998).

Thus, this study was carried out to evaluate four culture-based isolation protocols and to determine the optimal method for recovery of the *Arcobacter* species from cattle, beef and milk.

MATERIALS AND METHODS

Sample collection

A total of 142 samples including beef (n=52), fresh dairy cow milk (n=45) and rectal swabs from cattle (n=45) were collected. Beef samples were collected from six different retail markets, whereas rectal swabs and milk samples were collected from three dairy cattle farms, around Selangor, Malaysia. All the samples were collected between periods of April to July 2010. The samples were immediately transported to the laboratory in a container packed with ice and cultured within 3 to 4 h after sampling.

Sample preparation and isolation procedure

A total of 52 beef samples, were collected from various retail markets in the vicinity of Selangor and brought to Veterinary Public Health Laboratory in ice-packed containers. Ten grams of each sample was mixed with 90 ml of sterilized distilled water and homogenized in a stomacher. Thereafter 1 ml of homogenate was subjected 9 ml (to each) of four methods of isolation and incubated accordingly.

Rectal swab was collected by inserting a sterile cotton swab into the anus of animal, twisted (contacting the walls of rectum) and removed. The swabs were then placed individually into universal bottles containing appropriate enrichment broths, brought to the laboratory in ice box and incubated accordingly.

Fresh milk (100 ml) was collected from each animal and placed individually in sterile bottles and transported under cooled condition (4°C) to the laboratory and cultured within 3-5 h of collection. Milk samples were enriched (1:10) and incubated according to Method.

After incubation, in Method I, 20 µl of each enrichment broth culture was transferred by directly pipetting onto the surface of the agar plates. In Method II, III and IV, 100 µl of each enrichment broth culture was plated using membrane filters. The plates were incubated aerobically at 37°C for 1 h before the filters were removed. After filter removal, the plates were incubated at 30°C, under aerobic conditions for 48 h.

Isolation methods

Isolation methods and their modifications are detailed in Table 1: Method I (de Boer et al., 1996), Method II (Modified de Boer et al., 1996), Method III (Atabay and Corry, 1998) and Method IV (Modified Atabay and Corry, 1998) were used for *Arcobacter* detection and their sensitivity and specificity were compared. For Method II, the following modifications were made: enrichment broth was microaerobically incubated (condition generated by BD CampyPak^{TM}, Becton, Dickison and Company) at 30°C (instead of 24°C) for 48 h and then transferred on to Blood Agar Base no. 2 (Oxoid; CM0271) supplemented with 5% defibrinated horse blood and plates were kept aerobically at 30°C (not 24°C) for two days (not 5 days) and for inoculation onto agar medium, membrane filters of 47 mm diameter and 0.45 µ pore size (Sartorius, Ltd., Goettingen, Germany) were used. In Method IV, sheep blood was replaced with 5% defibrinated horse blood which was incorporated in plating agar medium.

Presumptive identification of isolates

Four to six whitish/gray, pin point, watery colonies from each plate were picked for Gram staining, catalase and oxidase tests. The organisms were also examined for motility, indoxyl acetate hydrolysis and hippurate hydrolysis activities (On et al., 2002; Atabay and Corry, 1998).

Confirmation of isolates by species specific multiplex PCR (mPCR)

For confirmation of isolates at species level, the mPCR technique (Houf et al., 2000) was used with some modifications in the agarose gel electrophoresis conditions. In brief, from each plate of purified isolate, a loopful of colonies was placed into 1 ml of sterilized distilled water (heavy suspension) in an Eppendorf tube (1.5 ml) for DNA extraction using DNA extraction kit (Promega, USA). For the simultaneous detection of *A. butzleri*, *A. cryaerophilus* and A. *skirowii*, the primers targeting 16S and 23S rRNA genes were used. A multiplex PCR reaction mixture of 50 µl contained 25 µl of 2x Master Mix (Qiagen, UK), 5 µl of 10x Primer mix (SKIR, BUTZ, ARCO, CRYI and CRYII), RNase free water and 2 µl of DNA extract. PCR conditions applied were as follows: All the DNA samples were pre-heated at initial Taq temperature of 95°C for 15 min followed by 32 cycles each of denaturation (94°C, 45s), primer annealing (61°C, 45s), chain extension (72°C, 30s) and final extension (72°C, 10 min). Amplified products were detected by electrophoresis in 1% agarose gel (80 V for 60 min). Finally, the gels were stained with gel red (Biotium, Hayward, CA) and examined under UV transilluminator. Reference strains of *A. butzleri* (CCUG 17812), *A. cryaerophilus* (CCUG 17801) and *A. skirrowii* (CCUG 30483) were used as positive controls; water served as the negative (no template) control.

Data analysis

The sensitivity and specificity of all methods were calculated using the following formulae (Merga et al., 2011).

$$\text{Sensitivity} = \frac{\text{No. of positive samples (by each method)}}{\text{Total no. of positive sample (by all methods)}}$$

$$\text{Specificity} = \frac{\text{No. of } \textit{Arcobacter} \text{ positive isolates (each method)}}{\text{No. of positive isolates + No. of non-}\textit{Arcobacter}\text{ (each method)}}$$

The differences in specificity and sensitivity of each isolation method were tested for significance using Fisher's exact test using

Table 1. Methods for isolation of *Arcobacter* spp. from beef, milk and cattle.

Method	Method I (MI) (de Boer et al., 1996)	Method II (MII) (Modified Method I)	Method III (MIII) (Atabay and Corry, 1998)	Method IV (MIV) (Modified Method III)
Enrichment broth Formulation[1]	***Arcobacter* selective broth** 28 g Brucella broth powder (Difco) 5% (vol/vol) lysed horse blood 75 mg piperacillin (Sigma) 32 mg cefoperazone (Sigma) 20 mg trimethoprim (Sigma) 100 mg cycloheximide (Serva)		**CAT broth**: 8 mg cefoperazone 10 mg amphotericin B 4 mg teicoplanin	
Incubation conditions	24°C for 48 h; aerobic	30°C; 48 h; microaerobic	30°C; 48 h; microaerobic	30°C; 48h; microaerobic
Plating medium Formulation[1]	***Arcobacter* selective medium** 21 g Muller Hinton broth (Oxoid) 2.5 g of agar no. 3 (Oxoid) 75 mg piperacillin (Sigma) 32 mg cefoperazone (Sigma) 20 mg trimethoprim (Sigma) 100 mg cycloheximide (Serva)	Blood Agar Base no. 2 (Oxoid) containing 5% defibrinated horse blood (without antibiotic supplements)	Blood Agar Base no. 2 (Oxoid) with defibrinated sheep blood	Blood Agar Base no. 2 (Oxoid) with 5% defibrinated horse blood added
Incubation conditions	24°C for 48-72 h; aerobic	30°C; 48 h; aerobic	30°C; 48 h; aerobic	30°C; 48 h; aerobic
Transfer of enriched broth culture onto agar plate	[3]Direct transfer (without membrane filtration)	[2]Membrane filtration	[2]Membrane filtration	[2]Membrane filtration
Estimated cost per sample (USD)	4.0	3.0	1.2	1.0
Total time required for isolation	5-6 days	4-5 days	3-4 days	3-4 days

[1]Quantities of the selected media and reagents are per liter of formulation.
[2]Membrane filtration: 100 µl of each enriched broth cultures were spotted onto cellulose acetate membrane filters (47 mm diameter, 0.45 µ pore size; Sartorius, Ltd., Goettingen, Germany) placed onto blood agar plates, with incubation for 1 h at 37°C.
[3]Direct transfer: 40 µl of each enriched broth culture was placed on the center of surface of ASM plate and incubated for 48-72 h at 24°C.

the GraphPad QuickCalcs free online calculator http://www.graphpad.com/quickcalcs/contingency1.cfm) . Results were considered as significantly different at P≤0.05.

RESULTS

The phenotypic characteristics revealed that the presumptive Arcobacter colonies were pin-point and translucent, showed Gram negative reaction and cork-screw type motility. Biochemically they were positive for catalase, oxidase and indoxyl acetate hydrolysis tests; *Arcobacter* spp. were

Table 2. Detection of *Arcobacter* spp. from beef samples using four different isolation methods.

Method	No. of samples positive	*Arcobacter* isolated	Sensitivity (%)	Specificity (%)
MI	7	7	43.7	70.0
MII	9	12	75.0	63.1
MIII	14	16	100.0	34.7
MIV	13	15	93.7	39.4
Total	16	--	--	--

--Not required for calculation.

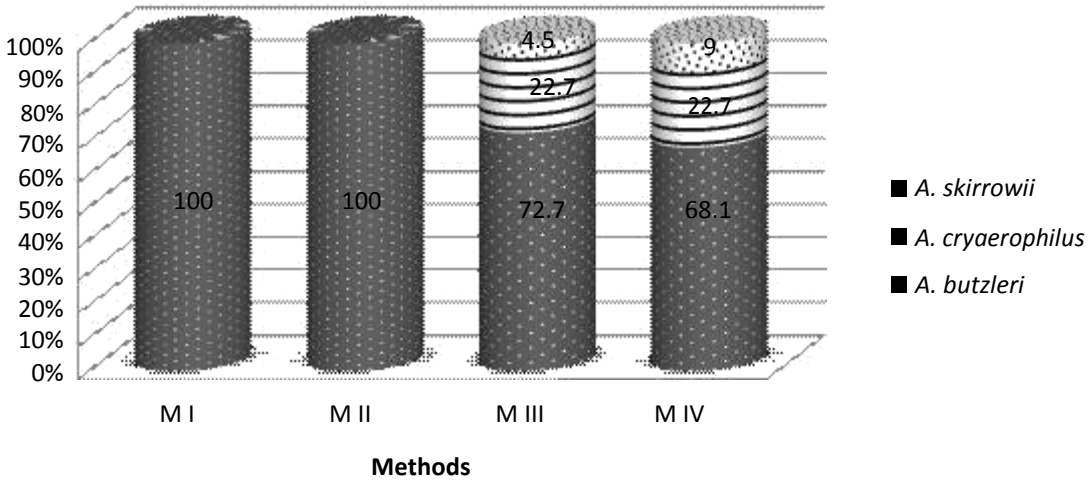

Figure 1. Speciation of Arcobacter detected from retailed beef.

Table 3. Detection of *Arcobacter* spp. from milk samples using four different isolation methods.

Method	No of samples positive	*Arcobacter* isolated	Sensitivity (%)	Specificity (%)
MI	0	0	0.0	0.0
MII	0	0	0.0	0.0
MIII	3	3	60.0	21.4
MIV	3	3	60.0	18.7
Total	**5**	--	--	--

--Not required for calculation.

negative for hippurate hydrolysis test.

The four isolation methods were compared for their ability to detect *Arcobacter* from beef (Table 2). MIII had the highest sensitivity (100%) among the four methods which was significantly different (P = 0.0030) when compared with MI, but was comparable to MII (P = 0.611) and MIV (P = 0.5000). Significant differences in sensitivity were also noted between M1 and MIV (P = 0.0004) and MII and MIV (P = 0.0138). On the other hand specificity of MI was highest (70%) compared to other methods and was significantly different (P = 0.0456) from MIII; MII was significantly different from MIII (P = 0.0341).

The discriminatory power of four methods for *Arcobacter* species was confirmed by using mPCR (Figure 1). MI and MII detected only *A. butzleri*, whereas MIII and MIV supported the growth of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. Of the total isolates detected from beef by MIII, 72.7, 22.7, 4.5% were positive for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively. The detection rate from beef using MIV was 68.1, 22.7, 9% *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively.

As shown in Table 3, *Arcobacter* was isolated from five of the 45 (11.11%) of the milk samples. MI and MII failed to detect any *Arcobacter* species from milk samples, whereas MIII and MIV were able to detect *Arcobacter*

Table 4. Detection of *Arcobacter* spp. from rectal swabs of dairy cattle using four isolation methods.

Method	No of samples positive	*Arcobacter* isolated	Sensitivity (%)	Specificity (%)
MI	0	0	0.0	0.0
MII	0	0	0.0	0.0
MIII	2	2	50.0	20.0
MIV	2	2	50.0	25.0
Total	4	--	--	--

-- Not required for calculation.

(60% each), however their specificity and sensitivity were non-significant (P = 0.1877 and P = 0.6046, respectively). *A. butzleri* was the only species isolated from all positive milk samples.

Similarly, MI and MII did not detect *Arcobacter* from rectal swabs of dairy cattle (Table 4). However, MIII and MIV detected *Arcobacter* but statistically their specificity and sensitivity were not significantly different (P = 0.6916 and P = 0.6176, respectively). Only *A. butzleri* was detected from all positive rectal swab samples.

DISCUSSION

MI and MII were quite cumbersome to perform and expensive because of the cost of antibiotics supplement. The recovery rate of *Arcobacter* by MI from beef was 7/16 (43.7%) and isolated only *A. butzleri* species; however MI did not detect *Arcobacter* from either milk or bovine rectal swabs. De Boer et al. Method I (1996) method could not detect any positive sample for *Arcobacter*, whereas the same samples were positive for *Arcobacter* when CAT (cefoperazone, amphotericin, teicoplanin) and membrane filtration method (MII) was used (Ongor et al., 2004). Furthermore, this method (MI) could not detect *Arcobacter* from *A. cryaerophilus*-positive samples (Houf et al., 2000). Various factors such as type and concentration of antimicrobial compounds in the media might influence the growth and isolation rate of *Arcobacter* (Atabay and Corry, 1998). *Arcobacter* spp. are susceptible to piperacillin and cefoperazone at concentrations slightly lower than those used in MI (Houf et al., 2001), which may have reduced the potential ofthe isolation protocol to support *Arcobacter* growth. The specificity of MI was highest (70%), which was probably due to addition of the antibiotic supplement.

Due to the sensitivity of *Arcobacter* species to antibiotics supplement, MI was modified by using cellulose acetate membrane filter instead of antibiotics supplement (MII) which improved the recovery rate (75%). The pore size of the membrane filter allows *Arcobacter* species to penetrate through (Atabay and Corry, 1998). Membrane filtration methods have been reported as superior to other isolation methods used so far (Engber et al., 2000).

For all samples examined, MIII was most sensitive in *Arcobacter* detection (100%). On the contrary its specificity was poor (34.7%) which may be due to the addition of blood, a universal ingredient to support many non-*Arcobacters*. *Arcobacter* enrichment broth containing CAT (cefoperazone, amphotericin, teicoplanin) antibiotic supplement tend to support the growth of *A. butzleri, A. skirrowii* and *A. cryaerophilus* species, but did not support the growth of *Campylobacter* strains (Philips, 2001), which was probably due to absence of oxygen-quenchers (such as blood) which neutralizes the toxicity of atmospheric oxygen (Atabay and Corry, 1998; Corry et al., 1995). The use of CAT broth followed by the passive filtration of the enriched broth culture (0.45 µm filters) on blood agar, has produced similar results to those obtained with direct detection by multiplex PCR (Collado et al., 2009a; 2008). *Arcobacter* species have also been recovered from beef (22%), lamb (15%) and chickens (73%) using medium containing CAT (Rivas et al., 2004). By using CAT-membrane filtration technique, 6.9% *Arcobacter* (*A. butzleri* 4%, *A. skirrowii* 2.9% and *A. cryaerophilus* 0.5%) from cattle rectal swabs were detected, whereas, 37% of minced beef (*A. butzleri*, 33% and *A. cryaerophilus* 3.7%) was found *Arcobacter* positive (Aydin et al., 2007).

The cost and difficulty in maintaining a ready supply of sheep blood for media preparation led to the development of MIV which replaced 5% defibrinated sheep blood with 5% defibrinated horse blood. This modification did not produce any significant difference (P=0.5000) when compared to MIII. On blood agar, *Arcobacter* produces large, round gray color colonies. *Arcobacter* can produce highly distinctive colony morphology (round gray colonies with characteristic pink color when picked with a white loop), which allows ready discrimination from non-*Arcobacters* (Scullion et al., 2004).

The ability of MIII and MIV to detect *Arcobacter* was better for beef, (87.5 and 81.2%), milk (60 and 40%) and cattle rectal swabs (40 and 40%), compared to MI and MII which detected *Arcobacter* in beef (43.7 and 56.2%, respectively) but not in either milk or cattle rectal swab samples.

The greatest problem encountered during the isolation of *Arcobacter* from meat samples was overgrowth of *Pseudomonas* and *Proteus*. *Proteus* species are commonly found on fresh meat and poultry (Jay, 1996) and

because of their sizes (approximately 0.4 to 0.8 µm in diameter and 1-3 µm in length) and motile nature they were able to cross the physical barrier of cellulose membrane filter (0.45 µm) (Holt et al., 1994). Moreover, a colony of *Arcobacter* was often completely overgrown by a rapid growth of competitive microflora. The plating media for *Arcobacter* are highly nutritious and many yeast species, especially *Candida* and *Trichosporon* were able to grow (Vytrasova et al., 2003).

Biochemical tests alone are not adequate to confirm *Arcobacter*, unless they are followed by PCR assay (Vytrasova et al., 2003). On confirmation of *Arcobacter* isolates by mPCR it was found that, MI and MII supported the growth of only *A. butzleri*. This may be due to addition of antibiotics supplement such as piperacillin and cefoperazone, which inhibited the growth of *A. skirrowii* and *A. cryaerophilus* (Houf et al., 2001). MIII and MIV supported *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. *Arcobacter* medium containing CAT supplement supported the growth of all three species of *Arcobacter* (Atabay and Corry, 1998).

In the present study, four isolation methods were compared to determine an optimal isolation method for *Arcobacter* from beef, milk and bovine rectal swabs. The detection rate of *Arcobacter* from various sources differed due to the sensitivity of methods. MI and MII were rather tedious to prepare and used costly materials. In addition, their detection and discriminatory potentials were not good as those of MIII and MIV, which detected highest number of positive samples and were able to discriminate *A. butzleri, A. cryaerophilus* and *A. skirrowii*.

ACKNOWLEDGEMENTS

The authors are indebted to Sindh Agriculture University Tandojam, Pakistan for Ph.D grant and University Putra Malaysia for financial support of the project (AgroDana). We also thank Dr. Goh Yong Meng, Faculty of Veterinary Medicine, for his assistance in statistical analysis.

REFERENCES

Atabay HI, Corry JEL (1998). Evaluation of a new *Arcobacter* enrichment medium and comparison with two media developed for enrichment of *Campylobacter* species. Int. J. Food Microbiol., 41: 53-58.

Aydin F, Fumussoy KS, Atabay HI, Ica T, Abay S (2007). Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J. Appl. Microbiol., 103: 27-35.

Collado L, Cleenwerck I, Stefanie VT, Paul DV, Figueras MJ (2009a). *Arcobacter* mytili sp. Nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels. Int. J. Syst. Evol. Microbiol., 59: 1391-1396.

Collado L, Inza I, Guarro J, Figueras MJ (2008). Presence of *Arcobacter* spp. in environmental waters correlates with high levels of fecal pollution. Environ. Microbiol., 10: 1635-1640

Collado L, Guarro J, Figueras MJ (2009b). Prevelance of *Arcobacter* in meat and shellfish. J. Food. Prot., 72: 1102-1106.

Corry JEL, Post DE, Colin P, Laisey MJ (1995). Culture media for the isolation of *Campylobacter*s. Int. J. Food Microbiol., 26: 43-76

De Boer E Tilberg, Woodward LJ, Johnson W (1996). A selective medium for the isolation of *Arcobacter* from meats. Lett. Appl. Microbiol., 23: 64–66.

De Smet S, De Zutter L, Van Hende J, Houf K (2010). *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. J. Appl. Microbiol., 108: 299-305

Ellis WA, Neill SD, O'Brien JJ, Hanna J (1978). Isolation of spirillum like organism from pig fetuses. Vet. Rec., pp. 102-106.

Ellis WA, Neill SD, O'Brien JJ, Ferguson HW, Hanna J (1977). Isolation of Spirillum/Vibrio-like organisms from bovine fetuses. Vet. Rec., 100: 451-452.

Engberg J, On SLW, Harrington CS, Gerner-Smidt P (2000). Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a re-evaluation of isolation methods for *Campylobacter*s. J. Clin. Microbiol., 38: 286–291.

Ertas N, Yusuf D, Zafer G, Ahmet G, Ismail U (2010). Prevalence of *Arcobacter* Species in Drinking Water, Spring Water, and Raw Milk as Determined by Multiplex PCR. J. Food Prot., 73(11): 2099-2102

Figueras MJ, Collado L, Levican A, Perez J, Solsona MJ, Yustes C (2010). *Arcobacter molluscorum* sp. nov., a new species isolated from shellfish. Syst. Appl. Microbiol., 34(2): 105-109

Gonzalez J, Botella S, Montes RM, Moreno Y, Ferrus MA (2007). Direct detection and identification of *Arcobacter* species by multiplex PCR in chicken and waste water samples from Spain. J. Food Prot., 70(2): 341-347

Harrab B, Schwarz S, Wenzel S (1998). Identification and characterization of *Arcobacter* isolates from broilers by biochemical tests, antimicrobial resistance patterns and plasmid analysis. J. Vet. Med., 45: 87–94.

Ho TKH, Lipman LJA, Wim G (2006). *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent. Int. J. Vet. Microbiol., 115: 1-13.

Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST (1994). Group 5: facultatively anaerobic gram negative rods, p. 184-185. *In:* Bergys' manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

Houf K, Stephan R (2007). Isolation and characterization of emerging foodborne pathogens *Arcobacter* from human stool. J. Microbiol. Methods, 68: 408-413.

Houf K, Tutenel A, De Zutter L (2000). Development of multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter* skirrowi and *Arcobacter cryaerophilus*. FEMS Microbiol. Lett., 193: 89–94

Houf K, Divriese LA, De Zutter L (2001). Susceptiblity of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* to antimicrobial agents used in selective media. J. Clin. Microbiol., 39: 1654-1656.

Houf K, On SLW, Coenye T, Debruyne L, Smet SD, Vandamme P (2009). *Arcobacter* thereius sp. nov., isolated from pigs and ducks. Int. J. Syst. Evol. Microbiol., 59: 2599-2604.,

Jacob J, Woodward D, Feuerpfeil I, Johnson WM (1998). Isolation of *Arcobacter butzleri* in raw water and drinking water treatment plants in Germany. Zentralbl. Hyg. Umweltmed., 201: 189-198.

Jay JM (1996). Modern Food Microbiology, 6th ed., APAC Publishers, Singapore.

Johnson LG, Murano EA (1999). Comparison of three protocols for the isolation of *Arcobacter* from poultry. J. Food Prot., 62: 610–614.

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

Merga JY, Leatherbarrow AJH, Winstanley C, Bennett M, Hart CA, Miller WG, Williams NJ (2011). Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter* spp. in Cheshire, United Kingdom. App. Environ. Microbiol., 1646–1650

Musamanno RA, Russi M, Lior M, Figura N (1997). In vitro virulence factors of *Arcobacter butzleri* strains isolated from supercicial water samples. Microbiologica, 20: 63-68

Neill SD, Ellis WA, O'Brien JJ (1979). Designation of aerotolerant *Campylobacter*-like organism from porcine and bovine abortion to the

genus *Campylobacter*. Res. Vet. Sci., 27: 180-186.

Ohlendorf DS, Murano EA (2002). Prevalence of *Arcobacter* spp. in raw ground pork from several geographical regions according to various isolation methods. J. Food Prot., 65: 1700–1705.

On SL, Jensen TK, Bille-Hansen V, Jorsal SE, Vandamme P (2002). Prevalence and diversity of *Arcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark. Vet. Microbiol., 85: 159-167

Ongor H, Cetinkaya B, Acik BN, Atabay HI (2004). Investigation of *Arcobacter*s in meat and fecal samples of clinically healthy cattle in Turkey. Lett. App. Microbiol., 38: 339–344.

Philips CA (2001). *Arcobacter* spp. in food: isolation, identification and control. Trends Food Sci. Technol., 12: 263–275.

Pianta C, Daniel TP, Diego H, de Oliveira SD (2007). Isolaion of *Arcobacter* spp. from the milk of dairy cows in Brazil. Ciencia Rural, Santa Maria, 37(1): 171-174.

Prouet-Mauleon V, Labadi L, Bouges N, Menard A, Megraud F (2006). *Arcobacter butzleri*: underestimated entropathogen. Emerg. Infect. Dis., 12: 307-309.

Rivas L, Fegan N, Vanderlinde P (2004). Isolation and characterization of *Arcobacter butzleri* from meat. Int. J. Food Microbiol., 91: 31–41.

Scullion R, Harrington CS, Madden RH (2004). Comparison of three methods for the isolation of *Arcobacter* spp. from retail raw poultry in Northern Ireland. J. Food Prot., 67(4): 799-804.

Scullion R, Clare SH, Robert MH (2006). Prevalence of *Arcobacter* species in raw milk and retail raw meats in Northern Ireland. J. Food Prot., 69(8): 1986-1990.

Shah AH, Saleha AA, Zunita Z, Murugaiyah M (2011). *Arcobacter*-An emerging threat to animals and animal origin food products? Trends Food Sci. Technol., 22: 225-236.

Skirrow MB (1994). Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. J. Com. Path., 111: 113-149.

Van Driessche E, Houf K, Vangroenweghe F, De Zutter L, Hoof JV (2005). Prevalence, enumeration and strain variation of *Arcobacter* species the feces of healthy cattle in Belgium. J. Vet. Microbiol., 105: 149-154.

Vandamme P, Falsen E, Rossau R, Hoste B, Segers P, Tygat R, De Ley J (1991). Revision of *Campylobacter*, *Helicobacter* and Wolinella taxonomy: emendation of generic description and proposal for *Arcobacter* gen. nov. Int. J. Syst. Bacteriol., 41: 88-103.

Vytrasova J, Pejchalova M, Harsova K, Binova S (2003). Isolation of *Arcobacter butzleri* and *A. cryaerophilus* in samples of meats and from meat processing plants by a culture technique and detection by PCR. Folia Microbiol., 48(2): 227-232.