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Prevalence of pathogenic *Arcobacter* species in South Korea: Comparison of two protocols for isolating the bacteria from foods and examination of nine putative virulence genes

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ABSTRACT

Contamination of foodstuffs by potentially enteropathogenic *Arcobacter* spp. is becoming a concern worldwide. However, few studies have examined virulence-associated genes in isolates of *Arcobacter* spp. from food. Here, we investigated the prevalence of three pathogenic *Arcobacter* species, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, in chicken, pork, and leafy green vegetables (n = 323) in South Korea. Samples were examined using two different protocols selected from a literature review: Acrobacter selective broth (ASB) II + Arcobacter selective medium (ASM) II (protocol A), and ASB II + modified charcoal cefoperazone deoxycholate agar supplemented with CAT (protocol B). Overall, *Arcobacter* spp. were detected in 45.8% of food samples, and the recovery rate of protocol B (37.8%) was significantly higher than that of protocol A (30.7%) ($p < 0.05$). Refrigerated chicken gizzard samples showed the highest detection rate (100%), followed by refrigerated chicken wing (79.5%), intestine (77.3%), neck skin (63.3%), pork (55.6%), frozen chicken legs (5.0%), and leafy green vegetables (4.4%) ($p < 0.05$). All isolates from chicken and leafy green vegetables were identified as *A. butzleri*, whereas *A. cryaerophilus* and *A. skirrowii* were mainly detected in pork. Most samples (95.8%) harbored more than one of nine putative virulence factors (*cadF*, *ciaB*, *cj1349*, *hecA*, *hecB*, *mviN*, *pldA*, *irgA*, and *tlyA*), and 91.3% harbored more than two. Isolates harboring all nine putative virulence genes were obtained from 1.9% of samples: five pork and one chicken. This study provides comprehensive and *de facto* evidence regarding prevalence of an emerging pathogen, *Arcobacter* spp., in various foods, along with their virulence potential. The results justify further research with respect to their role in food safety.

1. Introduction

The genus *Arcobacter* which, along with *Campylobacter*, belongs to the *Campylobacteraceae* family was first formed in 1991 (Vandamme et al., 1991). Since then, several studies of *Arocbacter* species have been published (Atabay and Corry, 1998; Collado and Figueras, 2011; On et al., 2002). At first, these organisms were thought to be commensal in humans and domestic animals (Vandamme, 2000); indeed, a number of studies report that *Arcobacter* spp. are present in water (Collado et al., 2008; Morita et al., 2004; Rice et al., 1999), in the feces and viscera of animals (e.g., broiler, cattle, horses, pigs, and sheep) (Collado and Figueras, 2011; Grove-White et al., 2014; Villalobos et al., 2013), and in slaughterhouses or processing lines (Shah et al., 2013; Tabatabaei et al., 2014); thus they are inhabitants of the natural environment.

To date, 28 *Arcobacter* species have been identified and characterized (Figueras et al., 2017; Hsu and Lee, 2015; Pérez-Cataluña et al., 2018; Ramees et al., 2017; Tanaka et al., 2017), six of which were isolated from mammals (Douidah et al., 2012). In particular, three species (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*), which can cause gastrointestinal disease in humans, are considered potentially enteropathogenic (Collado and Figueras, 2011; Ho et al., 2006; Levican et al., 2013). In 2002, the International Commission on Microbiological Specifications for foods (ICMSF) classified *Arcobacter* spp. as emerging foodborne pathogens (ICMSF, 2002). However, the occurrence of pathogenic *Arcobacter* in foods was underestimated because it was not routinely investigated due (mainly) to the absence of a standardized

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protocol for isolating bacteria from foods (Collado and Figueras, 2011; Vandenberg et al., 2004).

To the best of our knowledge, five enrichment broths and seven selective media have been proposed for selective growth and/or isolation of *Arcobacter* species from foods. The former are Ellinghausen-McCullough-Johnson-Harris Polysorbate 80 broth (EMJH P80) (Collins et al., 1996; Ellis et al., 1977), Arcobacter selective broth (ASB) I (de Boer et al., 1996), Johnson and Murano broth (JMB) (Johnson and Murano, 1999b), *Arcobacter* enrichment medium (AM) (Atabay and Corry, 1998), and ASB II (Houf et al., 2001). The latter arecephalothin, vancomycin, amphotericin agar (CVAA), modified cefsulodin, irgasan, novobiocin agar (mCINA) (Collins et al., 1996), ASM I (de Boer et al., 1996), JMA (Johnson and Murano, 1999b), ASM II (Houf et al., 2001), modified charcoal cefoperazone deoxycholate agar (mCCDA) with cefoperazone, amphotericin, and teicoplanin, and mCCDA with cefoperazone and amphotericin (Kemp et al., 2005; Merga et al., 2011). In addition, use of membrane filter on blood agar (MFBA) was also suggested as one of the protocols for selective isolation of *Arcobacter* species from foods (Atabay and Corry, 1997).

Several studies used different protocols for isolation of *Arcobacter* species from foods such as raw milk, raw/processed poultry and pork, shellfish, and vegetables (Atabay et al., 2003, 2006; Collado et al., 2009; Ferreira et al., 2013; González and Ferrús, 2011; Hausdorf et al., 2013; Houf et al., 2002; Laishram et al., 2016; Pejchalova et al., 2008; Rivas et al., 2004; Salas-Massó et al., 2016; Van Driessche and Houf, 2007). A few studies attempted to compare some of these methods using artificially inoculated samples (Fallas-Padilla et al., 2014; Johnson and Murano, 1999a; Merga et al., 2011; Scullion et al., 2004); however, only the study by Rathlavath et al. (2017a,b) compared the efficacy of the selected protocols with respect to confirming the presence of *Arcobacter* species in food.

Although the mechanism(s) underlying the pathogenicity and virulence of *Arcobacter* spp. in humans remains unclear, nine putative virulence-associated genes have been identified. Six putative virulence determinants, which are homologous to those of *C. jejuni*, encode fibronectin-binding proteins CadF and Cj1349, invasion protein CiaB, putative virulence determinant MviN, phospholipase PldA, and hemolysin TlyA (Miller et al., 2007). The other three virulence factors are genes encoding hemolysin activator protein HecB, a member of the filamentous hemagglutinin family (HecA), and the iron-regulated outer membrane protein IrgA (Douidah et al., 2012). Few studies have confirmed the presence of virulence-associated genes in *Arcobacter* spp. isolated from various sources, including animals, humans, and food (Collado et al., 2014; Ferreira et al., 2014; Girbau et al., 2015; Lehmann et al., 2015; Rathlavath et al., 2017a,b; Tabatabaei et al., 2014). In addition, there is a lack of comprehensive information about the prevalence and virulence of *Arcobacter* spp. in various types of foods.

Here, we isolated and identified three virulent species of *Arcobacter* spp. (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*) using two different protocols selected from a literature review and investigated their prevalence in chicken, pork, and leafy vegetables. In addition, we examined the potential pathogenicity of *Arcobacter* spp. isolated from Korean food stuffs by testing isolates for nine putative virulence-associated genes (*cadF*, *ciaB*, *cj1349*, *hecA*, *hecB*, *mviN*, *pldA*, *irgA*, and *tlyA*).

2. Materials and methods

2.1. Literature search and review to identify suitable protocols

To select appropriate protocols for detecting and/or isolating *Arcobacter* spp. from food materials, scientific publications with the following aims were identified: 1) to develop or suggest media for selective growth of *Arcobacter* spp.; 2) to compare known procedures for isolating *Arcobacter* spp. and evaluating their prevalence; or 3) to design PCR primers for genotyping or identifying virulence-associated genes in isolates. The experimental protocols used to detect or isolate *Arcobacter* spp. used in this study were selected by thoroughly reviewing and comparing all of the collected articles.

2.2. Sample collection

During August to October 2017, chicken, pork meat, and leafy green vegetables (lettuce, napa cabbage, spinach, or water parsley) were purchased from major supermarkets, online markets, or conventional markets in South Korea. Samples were selected based on statistics derived from Korean markets and the trade of food materials (KAPE, 2017). Collected samples comprised refrigerated whole chickens (n = 44), refrigerated chicken gizzard (n = 5), frozen chicken thighs or wings (n = 20), pork belly or neck (n = 90), and leafy green vegetables, including lettuce, napa cabbage, spinach, and water parsley (n = 90). Samples were placed individually in a cooler box immediately after collection and then transferred to the laboratory within 4 h.

2.3. Isolation of Arcobacter spp. from food

The results reported in section 2.1 allowed selection of a single protocol for selective enrichment of *Arcobacter* species, and of two selective media for isolation. Arcobacter selective broth (ASB) II (peptone 18.0 g, yeast extract 1.0 g, sodium chloride 5.0 g; Oxoid Ltd, Hampshire, UK) supplemented with amphotericin (10.0 mg/l), cefoperazone (16.0 mg/l), 5-fluorouracil (100.0 mg/l), novobiocin (32.0 mg/l), and trimethoprim (64.0 mg/l) (ASB II) was used for selective enrichment of *Arcobacter* species (Houf et al., 2001). All antibiotics were purchased from Sigma-Aldrich (St. Louis, MO, USA). The following media were used to isolate Arcobacter species from enriched samples: Arcobacter selective medium II, which is an ASB II containing 1.2% agar and 5% defibrinated horse blood (ASM II: protocol A); and modified charcoal cefoperazone deoxycholate agar (mCCDA) (nutrient broth (25.0 g), agar (12.0 g), charcoal (4.0 g), casein peptone (3.0 g), sodium deoxycholate (1.0 g); ferrous sulfate (0.25 g), sodium pyruvate (0.25 g); KisanBio Ltd, Seoul, Republic of Korea) supplemented with CAT supplement (Oxoid Ltd) containing cefoperazone (8.0 mg/l), amphotericin (10.0 mg/l), and teicoplanin (4.0 mg/l) (Kemp et al., 2005) (mCCDA with CAT: protocol B).

Using sterile forceps, 100 g of each sample was transferred to stomacher bags (Circulator 400 standard bags, Seward, Worthing, UK) containing 100 ml phosphate buffered saline (KisanBio Ltd) and homogenized for 2 min at 230 rpm (Circulator 400, Seward). ASB II (45 ml) was inoculated with 5 ml homogenate and mixed thoroughly using a vortex mixer at maximum speed. The mixture was then incubated aerobically at 30 °C for 48 h. One loopful of each enriched broth sample was streaked (in duplicate) on both ASM II and mCCDA/CAT and incubated aerobically at 30 °C for > 48 h. Two typical colonies selected from the ASM II (small, colorless, and convex with an intact edge) and mCCDA/CAT (small colorless or beige to off-white and flat with an entire edge) were selected and sub-cultured on blood agar (KisanBio Ltd.) supplemented with 5% defibrinated horse blood, and incubated at 30 °C for 48 h.

2.4. DNA preparation and PCR assay

Bacterial DNA was extracted from the sub-cultured cells from each isolate using a published boiling method, with some modifications (Kim et al., 2011; Lee et al., 2009). Briefly, one to three colonies of the bacterial isolate on blood agar were suspended in sterilized pure distilled water and boiled at 95 °C for more than 5 min. After cooling in an ice slurry, boiled cells were washed by centrifugation at 15,000 rpm for 5 min, and the supernatant, which contained debris, was discarded. Genomic DNA was extracted and used for PCR, and pure distilled water was used as the negative control. To identify the isolates, PCR was conducted using primer sets specific for *Arcobacter* genus and three pathogenic species (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*)

Table 1
Primers and PCR conditions used to identify *Arcobacter* genus and *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* species.

Primer name	Target gene	Nucleotide (5′ to 3′)	Product size (bp)	PCR conditions		No. of cycles	Reference
ARCO-F	23S rDNA	GTCGTGCCAAGAAAAGCCA	331	Denaturation	94 °C, 60 s	27	Bastyns et al. (1995)
ARCO-R		TTCGCTTGCGCTGACAT		Annealing	61 °C, 60 s		
				Extension	72 °C, 60 s		
BUTZ-F	16S rDNA	CCTGGACTTGACATAGTAAGAATGA	401	Denaturation	94 °C, 45 s	32	Houf et al. (2000)
BUTZ-R		CGTATTCACCGTAGCATAGC					
CRY-F	23S rDNA	TGCTGGAGCGGATAGAAGTA	257	Annealing	61 °C, 45 s		Houf et al. (2000)
CRY-R		AACAACCTACGTCCTTCGAC					
SKIR-F	16S rDNA	GGCGATTTACTGGAACACA	641	Extension	72 °C, 30 s		Houf et al. (2000)
SKIR-R		CGTATTCACCGTAGCATAGC					

Table 2
Primers used to detect putative virulence genes in *Arcobacter*.

Primer name	Target gene	Nucleotide (5′ to 3′)	Product size (bp)	PCR conditions		No. of cycles	Reference
ciaB-F	*ciaB*	TGGGCAGATGTGGATAGAGCTTGGA	284	Denaturation	94 °C, 45 s	32	Douidah et al. (2012)
ciaB-R		TAGTGCTGGTCGTCCCACATAAAG		Annealing	56 °C, 45 s		
cj1349-F	*cj1349*	CCAGAAATCACTGGCTTTTGAG	659	Extension	72 °C, 45 s		Douidah et al. (2012)
cj1349-R		GGGCATAAGTTAGATGAGGTTCC					
hecA-F	*hecA*	GTGGAAGTACAACGATAGCAGGCTC	537				Douidah et al. (2012)
hecA-R		GTCTGTTTTAGTTGCTCTGCACTC					
irgA-F	*irgA*	TGCAGAGGATACTTGGAGCGTAACT	437				Douidah et al. (2012)
irgA-R		GTATAACCCCATTGATGAGGAGCA					
mviN-F	*mviN*	TGCACTTGTTGCAAAACGGTG	294				Douidah et al. (2012)
mviN-R		TGCTGATGGAGCTTTTACGCAAGC					
cadF-F	*cadF*	TTACTCCTACACCGTAGT	283	Denaturation	94 °C, 45 s	32	Douidah et al. (2012)
cadF-R		AAACTATGCTAACGCTGGTT		Annealing	55 °C, 45 s		
hecB-F	*hecB*	CTAAACTCTACAAATCGTGC	528	Extension	72 °C, 30 s		Douidah et al. (2012)
hecB-R		CTTTTGAGTGTTGACCTC					
pldA-F	*pldA*	TTGACGAGACAATAAGTGCAGC	293				Douidah et al. (2012)
pldA-R		CGTCTTTATCTTTGCTTTCAGGGA					
tlyA-F	*tlyA*	CAAAGTCGAAACAAAGCGACTG	230				Douidah et al. (2012)
tlyA-R		TCCACCAGTGCTACTTCCTATA					

(Bastyns et al., 1995; Houf et al., 2000) (Table 1). In addition, the presence of nine putative virulence genes in each confirmed isolate was examined by PCR using specific primer sets (Douidah et al., 2012) (Table 2). All oligonucleotides were synthesized by Bioneer Ltd (Daejeon, South Korea).

The PCR protocols are described briefly in Tables 1 and 2. Prior to cycling, the PCR mixtures were heated to 94 °C for 2 min (initial denaturation); after cycling, the PCR mixtures were held at 70 °C for 3 min (final elongation). A thermal cycler (Bio-Rad Laboratory Inc., Hercules, CA, USA) was used for all experiments. Amplified products were detected by electrophoresis (Mupid®-One, Advance Co., Ltd, Tokyo, Japan) in 1.5% agarose gels run at 100 V for 30 min in 1 × Tris-acetate-EDTA (TAE) buffer. Gels were stained with a 1 × nucleic acid staining solution (Red Safe™, iNtRON Biotechnology Inc., Gyeonggi-do, South Korea) and visualized using Quantity One software (Bio-Rad) connected to a Universal Hood system II (Bio-Rad).

2.5. Statistical analysis

A Chi-squared test was performed to analyze the significance of the prevalence of *Arcobacter* spp. and the presence of the nine putative virulence genes in different food sources. A p value of < 0.05 was considered significant.

2.6. Reference strains

Type strains of *A. butzleri* (ATCC 49616, CAU 07646, 076048, 076050, and 080083),*A. cryaerophilus* (ATCC 43158), and *A. skirrowii* (ATCC 51132, CAU90017, 090019, and 090023) were used as positive controls (Wang et al., 2014).

3. Results and discussion

3.1. Selection of appropriate protocols to isolate Arcobacter species from food samples

As described above, previous studies identified five enrichment broths and seven selective media for isolation of *Arcobacter* spp. from foods. Several studies have compared these broths and media in terms of recovery rate and/or detection efficacy (selectivity and sensitivity). Johnson and Murano (1999a) suggested that the JMB + JMA combination (JM protocol) yields a higher recovery rate (84.0%) than the EMJH P80 + CVAA (48.0%) or ASB I + ASM I (30.0%) combinations. When others compared the JM protocol with AM + MFBA (Scullion et al., 2004), ASM II + ASM II (Scullion et al., 2004; Son et al., 2007), and ASB II + CVAA (Scullion et al., 2004), they deemed the JM protocol inefficient in terms of detecting *Arcobacter* spp. in foods because it generated over-growth of background flora, thereby reducing efficient detection of the target species.

According to Merga et al. (2011), the combination of ASB II + mCCDA with CAT showed the highest sensitivity (70.7%) and specificity (63.9%), followed by combinations of ASB II + ASM II (41.5% and 59.7% sensitivity and specificity, respectively), AM + ASM II (43.9% and 23.9%, respectively), AM + mCCDA with CAT (43.9% and 16.6%, respectively), and CEB + mCCDA with CA (43.9% and 43.2%, respectively). Fallas-Padilla et al. (2014) reported that ASB II + ASM II or MFBA showed higher specificity (90.0%) and sensitivity (89.3%) than ASB I + ASM II or MFBA (sensitivity, 21.4–35.7%; specificity, 50.0–84.0%). Therefore, we selected combinations recommended by previous studies (ASB II + ASM II (protocol A) and ASB II + mCCDA with CAT (protocol B)) to isolate *Arcobacter* spp. from food samples.

With respect to growth conditions, a number of early studies used

Table 3
Prevalence of *Arcobacter* in chicken, pork meat, and leafy green vegetables (bacteria isolated using two culture protocols).

Samples (number)	No. of positive samples (%)^{a}	No. of positive samples on ASM II (%) (protocol A)				No. of positive samples on mCCDA + CAT (%) (protocol B)			
		Arcobacter spp.	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*	*Arcobacter* spp.	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*
Refrigerated whole chicken									
Neck skin (n = 30)	19 (63.3) ^{d}	15 (50.0) ^{g}	15 (50.0)	–	–	16 (53.3) ^{h}	16 (53.3)	–	–
Wing (n = 44)	35 (79.5) ^{c}	22 (50.0) ^{g}	22 (50.0)	–	–	32 (72.7) ^{h}	31 (70.4)	1 (2.3)	–
Intestine (n = 44)	34 (77.3) ^{c}	27 (61.4)	27 (61.4)	–	–	27 (61.4)	27 (61.4)	–	–
Refrigerated chicken gizzard (n = 5)	5 (100.0) ^{b}	4 (80.0) ^{g}	4 (80.0)	–	–	2 (40.0) ^{h}	2 (40.0)	–	–
Frozen chicken portions									
Thigh/wing (n = 20)	1 (5.0) ^{f}	1 (5.0)	1 (5.0)	–	–	–	–	–	–
Pork meat (n = 90)	50 (55.6) ^{e}	29 (32.2) ^{g}	16 (17.8)	10 (11.1)	3 (3.0)	41 (45.6) ^{h}	18 (20.0)	22 (24.2)	1 (1.1)
Leafy green vegetables (n = 90)	4 (4.4) ^{f}	1 (1.1) ^{g}	1 (1.1)	–	–	4 (4.4) ^{h}	4 (4.4)	–	–
Total (n = 323)	148 (45.8)	99 (30.7) ^{g}	86 (26.6)	10 (3.1)	3 (0.9)	122 (37.8) ^{h}	98 (30.3)	23 (7.1)	1 (0.3)

[a]Detection rate of *Arcobacter* in each food sample.
[b–f]Numbers in the same column but denoted by different superscript letters are significantly different ($p < 0.05$).
[g–h]Data in the same row denoted by different superscript letters in the same row are significantly different ($p < 0.05$).

microaerobic conditions (Collado and Figueras, 2011); however, recent studies suggest that aerobic incubation, rather than microaerobic conditions, results in better recovery of *Arcobacter* species (Levican et al., 2014; Merga et al., 2011; Son et al., 2007). Thus, to achieve the highest possible detection and recovery rates, we incubated samples under aerobic conditions.

3.2. Prevalence of Arcobacter spp. in foodstuffs in South Korea

Table 3 shows the prevalence of *Arcobacter* in chicken, pork meat, and leafy green vegetables in South Korea. *Arcobacter* spp. was isolated from 148 food samples, resulting in an overall detection rate of 45.8%. Among collected samples, *Arcobacter* spp was most common in refrigerated chicken meat (63.3–100.0% of samples), followed by pork meat (55.6%), frozen chicken portions (5.0%), and leafy green vegetables (4.4%) ($p < 0.05$). In particular, all refrigerated chicken gizzard samples contained *A. butzleri*. More than 60% of refrigerated whole birds contained *Arcobacter* spp., and all were identified as *A. butzleri* (except one isolate from a chicken wing). In addition, detection rates in wing (79.5%) and intestine (77.3%) were significantly higher than those in neck skin (63.3%) ($p < 0.05$). This may infer that exposure of chicken meat to the giblets, which are mechanically removed during slaughter, increases the risk of contamination with *Arcobacter* spp. More than half of pork meat samples (n = 50, 55.6%) contained *Arcobacter* spp., comprising *A. butzleri* (n = 26, 28.9%), *A. cryaerophilus* (n = 24, 26.7%), and *A. skirrowii* (n = 4, 4.4%).

Since the early 2000s, *Arcobacter* species have been detected in raw milk, raw/processed meat, and shellfish worldwide, including Australia (Rivas et al., 2004), Belgium (Houf et al., 2002; Van Driessche and Houf, 2007), China (Hsu and Lee, 2015), the Czech Republic (Pejchalova et al., 2008), Denmark (Atabay et al., 2006), Germany (Hausdorf et al., 2013), India (Laishram et al., 2016), Ireland (Scullion et al., 2006), Japan (Tanaka et al., 2017), Portugal (Ferreira et al., 2013), Spain (Collado et al., 2009; González and Ferrús, 2011), and Turkey (Atabay et al., 2003). Although several research teams have attempted to isolate *Arcobacter* species from food stuffs, no data concerning the prevalence of *Arcobacter* species in food are available in most countries (exceptions include some of the countries mentioned above).

A number of previous studies report broad ranges of *Arcobacter* spp. prevalence in chicken and pork meat (12.0–86.0% and 'not detected' to 53.0%, respectively) (Amare et al., 2011; Fallas-Padilla et al., 2014; Fernandez et al., 2015; Ferreira et al., 2013; Lee et al., 2010; Molva and Atabay, 2016; Nieva-Echevarria et al., 2013; Patyal et al., 2011; Zacharow et al., 2015) and in leafy green vegetables (9.8–38.1%) (González et al., 2017; Mottola et al., 2016). The prevalence of *Arcobacter* spp. in chicken (63.3–100.0%) and pork (55.6%) meat reported herein is greater than that in these previous reports, while that in leafy green vegetables is lower (4.4%). In particular, whereas Lee et al. (2010) reported that *Arcobacter* species are present in only 22.2% of chicken samples and no pork meat samples, the data presented herein suggest a much higher prevalence of *Arcobacter* species in these meats in South Korea.

When we compared the detection rates after the two culture protocols used herein, we found that the detection rate after protocol B was significantly higher (37.8%) than that after protocol A (30.7%) ($p < 0.05$). In particular, protocol B (mCCDA with CAT) worked better than protocol A (ASB II) for detecting *Arcobacter* species in neck skin and wings of refrigerated whole chicken, pork meat, and leafy green vegetables ($p < 0.05$). However, protocol B did not always result in more effective recovery of *Arcobacter* spp. from food samples than protocol A because there were a significant number of cases in which only protocol A isolated *Arcobacter* spp. from chicken (three neck skin samples, 10%; three wing samples, 6.8%; five intestine samples, 11.4%; three gizzard samples, 60.0%; one frozen leg sample, 5.0%) and pork meat samples (two belly samples, 2.2%).

These results are comparable with those presented in the most recently published version of Bergey's Manual of Systematics of Archaea and Bacteria about *Arcobacter* (Vandamme et al., 2015). They report that mCCDA does not support growth of *Arcobacter* due to their sensitivity to the inhibitory substances present in the medium and/or the synergistic inhibitory effects of inhibitors (cefoperazone and sodium deoxycholate). The original composition of the antibiotic supplement for mCCDA was cefoperazone (32.0 mg/l), amphotericin B (2.0 mg/l), and rifampicin (10.0 mg/l). Although we did not use rifampicin, the concentrations of amphotericin B used in protocol A and B (4.0 and 10.0 mg/l, respectively) were much higher than those in the original composition (2.0 mg/l), and the concentrations of cefoperazone (16.0 and 8.0 mg/l) were much lower (original, 32.0 mg/l). The concentration of cefoperazone in protocol B using mCCDA as a basal medium was only one fourth that in the original composition; this might be one of the reasons why protocol B yielded a better recovery rate overall.

Many publications report that *Arcobacter* spp. (particularly three of the target species examined herein [*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*]) are resistant to 64 mg/l of cefoperazone; thus this phenotypic characteristic is sometimes used to identify *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* (Collado and Figueras, 2011; On and Holmes, 1991; On, 1996; Vandamme et al., 2005, 2015). However, the results of the present study suggest that the concentration of cefoperazone has a significant effect on the recovery of *Arcobacter* spp. from food, and that

Table 4
Presence of virulence determinants in the *Arcobacter* strains isolated in this study.

Species	Sources		No. of strains	Strains generating a virulence-associated gene amplicon (number (%))								
				cadF	*ciaB*	*cj1349*	*hecA*	*hecB*	*irgA*	*mviN*	*pldA*	*tlyA*
A. butzleri	Chicken	Refrigerated neck skin	49	16 (32.7)	30 (61.2)	22 (44.9)	6 (12.2)	9 (18.4)	6 (12.2)	36 (73.5)	41 (83.7)	42 (85.7)
		Refrigerated wing	86	26 (30.2)	63 (73.3)	56 (65.1)	7 (8.1)	10 (11.6)	7 (8.1)	53 (61.6)	75 (87.2)	76 (88.4)
		Refrigerated intestine	91	22 (24.2)	55 (60.4)	61 (67.0)	14 (15.4)	13 (14.3)	8 (8.8)	64 (70.3)	77 (84.6)	82 (90.1)
		Refrigerated gizzard	10	8 (80.0)	5 (50.0)	7 (70.0)	–	–	–	7 (70.0)	9 (90.0)	10 (100.0)
		Frozen thigh	1	–	–	–	–	–	–	–	1 (100.0)	1 (100.0)
	Pork meat	Belly	41	16 (39.0)	35 (85.4)	25 (61.0)	13 (31.7)	10 (24.4)	7 (17.1)	31 (75.6)	40 (97.6)	39 (95.1)
		Neck	8	2 (25.0)	6 (75.0)	6 (75.0)	3 (37.5)	3 (37.5)	2 (25.0)	6 (75.0)	8 (100.0)	8 (100.0)
	Leafy green	Napa cabbage	2	1 (50.0)	–	1 (50.0)	–	–	–	1 (50.0)	2 (100.0)	2 (100.0)
		Water parsley	8	3 (37.5)	6 (75.0)	4 (50.0)	1 (12.5)	–	2 (25.0)	7 (87.5)	6 (75.0)	6 (75.0)
A. cryaerophilus	Pork meat	Belly	36	3 (8.3)	25 (69.4)	16 (44.4)	12 (33.3)	6 (16.7)	7 (19.4)	27 (75.0)	20 (55.6)	19 (52.8)
		Neck	8	–	4 (50.0)	1 (12.5)	–	–	–	5 (62.5)	4 (50.0)	4 (50.0)
A. skirrowii	Pork meat	Belly	6	–	2 (33.3)	–	–	–	–	5 (83.3)	–	–
Total			346	97 (28.0)	231 (66.8)	199 (57.5)	56 (16.2)	51 (14.7)	39 (11.3)	242 (69.9)	283 (81.8)	289 (83.5)

over-use of cefoperazone (a range > 8.0–16.0 mg/l) to effectively suppress natural flora in the media may inhibit growth of the target organisms (*Arcobacter* spp.). In addition, it may suggest that selective recovery of *Arcobacter* spp. from food samples may be hampered by three of the other antibiotic agents (5-fluorouracil (100.0 mg/l), novobiocin (32.0 mg/l), and trimethoprim (64.0 mg/l)) added to ASM II in protocol A.

3.3. Presence of putative virulence genes in Arcobacter isolates

Overall, 361 *Arcobacter* strains were isolated from food samples collected in South Korea. Almost all (n = 346, 95.8%) harbored more than one virulence-related gene; the exceptions were seven strains of *A. butzleri* and eight strains of *A. cryaerophilus* isolated from chicken and/or pork meat (data not shown). Table 4 shows the distribution of virulence determinants in *Arcobacter* strains (n = 346) harboring more than one virulence-related gene. Most (n = 316, 91.3%) harbored more than two virulence-associated genes; the exceptions were 30 strains harboring only *mviN* (n = 21), *ciaB* (n = 7), or *hecA* (n = 2) (data not shown). Only six isolates (1.9%) harbored all putative virulence genes (four pork belly samples, one pork neck sample, and one chicken intestine sample).

Among the nine putative virulence determinants, the hemolysin gene (*tlyA*) was the most commonly identified in *Arcobacter* isolates (83.5%), followed by the phospholipase gene (*pldA*; 81.8%), the virulence factor *mviN* (69.9%), and the *Campylobacter* invasive antigen B gene (*ciaB*; 66.8%) (Table 4). Detection rates of genes encoding fibronectin-binding proteins (*cj1349*and *cadF*), a member of the filamentous hemagglutinin family gene (*hecA*), a hemolysin activation protein (*hecB*), and an iron-regulated outer membrane protein (*irgA*) were 57.5%, 28.0%, 16.2%, and 14.7%, respectively.

These results show partial agreement with those of previous studies performed in Belgium, Chile, Iran, Germany, and Spain (Collado et al., 2014; Douidah et al., 2012; Ferreira et al., 2014; Girbau et al., 2015; Lehmann et al., 2015; Tabatabaei et al., 2014). These other studies reported that six putative virulence determinants (*cadF*, *ciaB*, *cj1349*, *mviN*, *pladA*, and *tylA*) were identified more frequently than the others (*hecA*, *hecB*, and *irgA*) in *Arcobacter* isolated from various sources. Although these studies report that almost all *A. butzleri* isolates harbored six frequent putative virulence genes, the results of the present study indicate widely diverse detection rates (24.2–100.0%). Contrary to previous results, we found that the six putative virulence genes did not always co-exist, which may imply that *Arcobacter* harboring more variant genotypic patterns could be present in a variety of food samples worldwide.

In conclusion, we examined the prevalence of three well-known pathogenic members of *Arcobacter* species (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*) in chicken, pork meat, and leafy green vegetables in South Korea. The results show higher detection rates than reported by previous studies. As far as we are aware, this is the first study to confirm the presence and distribution of nine putative virulence determinants in *Arcobacter* spp. isolated from a variety of foodstuffs marketed in South Korea. The results support the idea that these foods are a possible route of transmission for a potential human enteropathogen. However, to confirm the magnitude of the potential hazard, the actual roles played by these genes in each strain need to be elucidated, as does the number of virulent species harboring these virulence-associated genes. Therefore, more in-depth and promising approaches are needed to detect virulent strains of *Arcobacter* spp. and to examine their virulence (*in vitro and in vivo*) in detail.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fm.2018.09.008.

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