Occurrence of *Aliarcobacter* spp. in fresh and pre-cut vegetables of common use in San José, Costa Rica

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Abstract

Aliarcobacter is a Gram-negative rod that can cause disease in both animals and humans. Several studies have evidenced its presence in a wide variety of foods. Given that the number of foodborne illness outbreaks linked to the consumption of vegetables has increased worldwide and that there is a lack of information about the occurrence of *Aliarcobacter* spp. in these, the aim of this study was to evaluate its presence and the occurrence of virulence factors in both fresh and ready-to-eat vegetable samples. 180 vegetable samples from Costa Rica were analyzed for the presence of *Aliarcobacter* spp., including 90 pre-cut vegetable packages and 90 fresh vegetables. Two (2.2%) of the isolates from pre-cut vegetables and 19 (21.1%) of the isolates obtained from fresh vegetables were confirmed as *Aliarcobacter* spp. One of the isolates from the pre-cut vegetable samples was identified as *A. butzleri*. For the fresh vegetables, 11 isolates were identified as *A. skirrowii*, one as *A. butzleri*, and the 7 remaining isolates could not be identified at a species level. There is an 87.5% positivity for *hecA* and 93.8% for *pldA*, virulence genes in strains isolated from fresh produce, contrasting with an absolute absence from pre-cut vegetable-isolated strains. These results evidence the presence of *Aliarcobacter* on fresh and pre-cut vegetables from Costa Rica and the potential hazard it might represent for public health.

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Introduction

Aliarcobacter is a genus of Gram-negative curved rods with polar flagella, derived from the genus *Arcobacter*. Recently, based on 16S rRNA identity, the *Arcobacter* genus was emended as the *Aliarcobacter* genus, including the species *Aliarcobacter cryaerophilus*, *Aliarcobacter butzleri*, *Aliarcobacter skirrowii*, *Aliarcobacter thereius*, *Aliarcobacter trophiarum*, *Aliarcobacter cibarius*, *Aliarcobacter lanthieri,* and *Aliarcobacter faecis* (Perez-Cataluña *et al.,* 2018, 2019). It can be distinguished from *Campylobacter* due to its ability to grow below 30°C (growth range 15-42°C), and its tolerance to aerobic conditions (Vandamme *et al*., 1991; Shah *et al*., 2011; Calvo *et al*., 2013; Ramees *et al*., 2017). This bacteria is rapidly inactivated above 55°C (D'sa and Harrison, 2005; Ramees *et al*., 2017). *Aliarcobacter* strains can tolerate a wide range of pH from 5.5 to 9.5 but optimal growth occurs between pH 6.8-8.0 (Neill *et al*., 1979; Ramees *et al*., 2017).

Aliarcobacter spp. can cause disease in both animals and humans; therefore, it has zoonotic potential. In animals, *Aliarcobacter* spp. have been reported in diarrhea, mastitis, and abortions. In humans, it can lead to septicemia, endocarditis, peritonitis, gastroenteritis, and diarrhea. *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. thereius* have been recognized as human pathogens (Araya-Quesada *et al*., 2014; Simaluiza *et al.,* 2021). In Costa Rica, Barboza *et al*. (2017) described the first case of diarrhea due to an infection by *A. cryaerophilus* in the country.

Among the virulence factors that have been described in pathogens of this genus, the 4 main ones that have been studied are adhesion, invasion of host cells, production and secretion of toxins, and induction of a pro-inflammatory environment by cytokines (Collado and Figueras, 2011; Ferreira *et al*., 2016; Ramees *et al*., 2017). In turn, the pathogenicity mechanisms have been investigated with the intention of better characterizing *Aliarcobacter*. A total of 9 putative genes have been mostly associated with these processes: *cadF*, *ciaB*, c*j1349, mviN, pldA, tlyA, irgA, hecA,* and *hecB* (Collado and Figueras, 2011; Ferreira *et al*., 2016; Ramees *et al*., 2017).

Several studies on a wide variety of foods have been able to evidence the presence of *Aliarcobacter* spp. It has been isolated

from poultry, pork, goats, sheep, beef cattle, and rabbits; additionally, it has been reported in milk, mollusks, and vegetables (Nieva-Echavarría *et al.,* 2013; Ramees *et al*., 2017; Ferreira *et al.,* 2019). Calvo *et al.* (2013) emphasize that among meat products, it is most prevalent in chicken meat, followed by pork meat and beef meat. In Portugal, its prevalence in retail foods was analyzed; the results showed a prevalence of 47.6% in ready-to-eat vegetables, 92% in poultry meat, 45.8% in pork meat, 42.1% in beef meat, and 68% in fish (Vicente-Martins *et al.*, 2018). A similar study conducted by Molva and Atabay (2016) with retail poultry meat in Turkey found this microorganism in all the specimens analyzed.

Given that the number of foodborne illness outbreaks linked to the consumption of fresh vegetables and ready-to-eat vegetables has increased worldwide and that there is an important lack of information about the occurrence of *Aliarcobacter* spp. in vegetables, the aim of this study was to evaluate its presence and the occurrence of virulence factors of this bacterium isolated from both fresh and ready-to-eat vegetable samples.

Materials and Methods

Aliarcobacter spp. isolation

A total of 180 vegetable samples were analyzed for the presence of *Aliarcobacter* spp.: 90 pre-cut vegetable packages purchased at supermarkets and 90 fresh vegetables purchased from farmers' markets in Costa Rica. Both the pre-cut vegetables and the fresh vegetables were purchased as follows: 30 units of spinach, 30 units of arugula, and 30 units of lettuce. 30 g of each sample were placed in sterile plastic bags with 100 mL of sterile peptone water 0.1% and were mixed and stomached afterward. A total volume of 1 mL was then inoculated in Houf broth (Oxoid®, Basingstoke, UK) and incubated at 35°C for 24 hours. Isolation was achieved by means of the membrane filter method, which is regularly used in our laboratory. In brief, a 47 mm diameter and 0.45 m pore triacetate sterile membrane filter was placed on sheep blood agar plates, and various drops (10-12) of the enrichment media were placed onto the membrane filter. The membrane was removed after filtration and incubated at 26°C under aerobic conditions for 48 hours. After the incubation period, typical colonies were isolated and plated in blood agar until pure cultures were obtained. Typical colonies in blood agar are described as brilliant, non-pigmented, round, 1-2 mm in diameter, convex, and full border, translucid or with a slight gray dye. Because this research was based on the determination of the presence frequency of *Aliarcobacter* spp. on fresh and pre-cut vegetables, DNA coming from the cloning of one characteristic colony was extracted per sample.

DNA extraction

A DNA extraction was used to later identify the isolates by polymerase chain reaction (PCR) and multiplex PCR (genus and species, respectively). For the extraction, 1 mL of sterile saline solution and one colony were placed in microcentrifuge tubes. The tubes were boiled in a water bath for 10 minutes. They were then centrifuged at 3000 rpm for 5 minutes. The supernatant was pipetted into another microcentrifuge tube and frozen for further analysis.

Aliarcobacter genus identification by polymerase chain reaction

A PCR was then employed, as described by Harmon and Wesley (1996), for genus identification. A gene-specific RNAr fragment was amplified using a forward primer Arco I (5′-AGA GAT TAG CCT GTA TTG TAT C-3'), and a reverse primer Arco II (5′-TAG CAT CCC CGC TTC GAA TGA-3′). To analyze the PCR products, gel electrophoresis was used with an agarose gel 1.5%, and the results were considered positive if a 1223 bp fragment was obtained.

Species identification by multiplex polymerase chain reaction

To identify the isolates on a species level, a multiplex PCR was used, as described by Douidah *et al.* (2012). The primers used are presented in Table 1. Amplification was performed in an automatic thermocycler with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1 minute, and a final elongation at 72°C for 5 minutes. The PCR products were separated by electrophoresis in an agarose gel 2% and visualized with ultraviolet light.

Virulence genes determination

The following virulence genes were analyzed by PCR using the respective forward and reverse primers according to the method described by Douidah *et al*. (2012): c*adF, ciaB, cj1349, mviN, pldA, tlyA, irgA, hecA* and *hecB*. PCR involved 32 cycles of denaturation (94°C, 45 seconds); primer annealing at 56°C for 45 seconds for the primer sets for *ciaB*, *cj1349*, *hecA*, *irgA*, and *mviN* and at 55°C for 45 seconds for the primer sets for *cadF*, *hecB*, *pldA*, and *tlyA*; and a chain extension (72°C, 45 seconds) followed by final elongation for 3 minutes at 72°C.

Table 1. Primers used for the species identification polymerase chain reaction.

Species	Forward primer	Sequence	Reverse primer	Sequence	bp
Aliarcobacter butzleri	Arco F	5′-GCT AGA GGA AGA GAA ATC AA-3′	ButR	5′-TCC TGA TAC AAG ATA ATT GTA CG-3′	2061
Aliarcobacter thereius	Arco F	5′-GCT AGA GGA AGA GAA ATC AA-3′	TherR	5′-GCA ACC TCT TTG GCT TAC GAA-3′	1590
Aliarcobacter cibarius	Arco F	5′-GCT AGA GGA AGA GAA ATC AA-3′	CibR	5′-CGA ACA GGA TTC TCA CCT GT-3′	1125
Aliarcobacter skirrowii	Arco F	5′-GCT AGA GGA AGA GAA ATC AA-3′	SkiR	5′-TCA GGA TAC CAT TAA AGT TAT TGA TG-3′	198
Aliarcobacter cryaerophilus	CriF	5′-CAG AGG AAG AGA AAT CAA AT-3′	CriR	5′-CCC ACT ATT CCA TCA GTG AG-3′	395

Results

Detection of *Aliarcobacter* spp. in suspicious isolates by polymerase chain reaction for genus confirmation

Out of all the analyzed vegetables, 7/90 pre-cut vegetables and 21/90 fresh vegetables presented suspicious colonies for the bacterium of interest (Gram-negative, small, and colorless colonies). The presumptive colonies were then confirmed by genus PCR; 2 (2.2%) of the isolates from pre-cut vegetables were confirmed as *Aliarcobacter* spp. and 19 (21.1%) of the isolates obtained from fresh vegetables were also confirmed as *Aliarcobacter* spp. The results showed that the prevalence of *Aliarcobacter* spp. in fresh vegetables is different, being almost 10 times higher (21.1%) than the prevalence in pre-cut vegetables (2.2%).

Among the fresh vegetables studied, arugula had the greatest prevalence of *Aliarcobacter* spp. 12/30 (40%), followed by lettuce 5/30 (16%) and spinach 4/30 (13%). In the pre-cut vegetables, there were no positive spinach samples, while lettuce and arugula had the same prevalence of 1/30 (3%).

Detection of *Aliarcobacter* species in isolates by multiplex polymerase chain reaction

When analyzing the *Aliarcobacter* spp. isolates by multiplex PCR for species identification, it was possible to identify one of the isolates from the pre-cut vegetable samples as *A. butzleri,* while the other isolate could not be identified to a species level. For the fresh vegetables, 11 isolates were identified as *A. skirrowii*, one isolate was identified as *A. butzleri*, and the 7 remaining isolates could not be identified at species level.

Identification of virulence genes in isolates of *Aliarcobacter* species by polymerase chain reaction

All the isolated strains had at least 4 of the analyzed virulence genes, as shown in Table 2. Although the number of isolates to compare is quite different, it is important to highlight an 87.5% positivity for *hecA* and 93.8% for *pldA* virulence genes in strains isolated from fresh produce, contrasting with an absolute absence from pre-cut vegetable isolated strains. Also, when comparing the presence of virulence genes on the *A. butzleri* isolates, 7/9 were identical; nevertheless, the pre-cut vegetable *A. butzleri* isolate presented *hecA* and no *cj134*, contrasting with the fresh produce isolate.

Discussion and Conclusions

The number of foodborne diseases has increased in the last few years, representing a serious challenge for health agencies and the food industry (Hausdorf *et al.*, 2013; Lake *et al.,* 2018). Raw fruits and vegetables have been known to serve as vehicles for various foodborne pathogens worldwide, a fact associated with the use of poor-quality water irrigation systems and pre-harvest steps (Ferreira *et al*., 2016). Vegetables can also be contaminated directly by fecal discharges from infected animals (Hausdorf *et al.*, 2011). Also, vegetables' complex surface and porosity facilitate pathogen attachment and survival (Mottola *et al*., 2016).

Aliarcobacter is a zoonotic, foodborne pathogen associated with diarrheal symptoms in humans and animals worldwide (Ramees *et al.*, 2017). Its isolation has been achieved from different food sources, including chicken, pork, beef, shellfish, water,

Table 2. Virulence genes found in isolates of Aliarcobacter species isolated from vegetables.

Vegetable	Species	Gene								
		hecA	*ciaB*	*cadF*	*hecB*	*tlyA*	*cj1349*	*mviN*	*pldA*	*irgA*
Fresh lettuce	*Aliarcobacter skirrowii*	+	+	+	+				+	
Fresh lettuce	*Aliarcobacter skirrowii*	+				+	+	+	+	
Fresh lettuce	*Aliarcobacter butzleri*	+	+	+		+		+		+
Fresh spinach	*Aliarcobacter skirrowii*	+	+	+	+	+	+	+	+	+
Fresh spinach	*Aliarcobacter* sp.	+	+	+		+	+	+	+	+
Fresh spinach	*Aliarcobacter* sp.	+	+	+		+	+	+	+	+
Fresh spinach	*Aliarcobacter* sp.	+	+	+		+	+	+	+	+
Fresh Arugula	*Aliarcobacter* sp.		+	+		+	+	+	+	+
Fresh Arugula	*Aliarcobacter* sp.					+		+	+	+
Fresh Arugula	*Aliarcobacter* sp.	+	+	+	+	+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*	+	+	+		+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*	+		+		+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*	+		+		+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*	+	+	+	+	+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*	+	+	+	+	+	+	+	+	+
Fresh Arugula	*Aliarcobacter skirrowii*			+	+	+	+	+	+	+
Total n(%)		14 (87.5)	11 (78.6)	14 (87.5)	6 (42.9)	15 (93.8)	13 (81.3)	15 (93.8)	15 (93.8)	14 (87.5)
Pre.cut Arugula	*Aliarcobacter butzleri*		+	+		+	+	+		+
Pre-cut lettuce	*Aliarcobacter* sp.			+	+	+	+	+		+
Total n(%)		0	1 (50)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	0	2 (100)

and vegetables. Very little is known about the prevalence of this bacteria in vegetables and fruits because most studies have focused on foods of animal origin; nevertheless, it has been isolated from broccoli, carrots, celery, cauliflower, lettuce, mushrooms, spinach, and tomatoes, as well as from fruits including apples, grapes, kiwis, strawberries, and watermelon (Winters and Slavik, 2000).

In the present study, the isolation of *Aliarcobacter* spp. from fresh and ready-to-eat vegetables is described. A greater prevalence in fresh vegetables (21.1%) than in pre-cut vegetables (2.2%) was found, which is justified by the fact that pre-cut vegetables are previously processed. These foods carry within their processing stages of inspection, selection, washing, cooling, size reduction (peeling, cutting, and grating), disinfection, and packaging, during which it is intended to guarantee that it is a ready-to-eat product. However, as shown by the results, this process does not eliminate 100% of the *Aliarcobacter* species present in the vegetables.

The survival of *A. butzleri* within several sanitizing food processing environments, including the use of chlorine, ethanol, and sodium chloride at different concentrations, has been demonstrated, as has the fact that chilled temperatures do not act as a barrier for this bacteria (Ferreira *et al.*, 2016). A total of 21 isolates were found: 2 in pre-cut vegetables, including 1 *A. butzleri*, and 19 in fresh vegetables. 12 of these isolates were identified at species level. The most frequent species found was *A. skirrowii*, followed by *A. butzleri*. This is particularly important because both species have been recognized as human pathogens. The remaining 7 isolates likely belong to non-pathogenic species or might represent species not yet described.

Similar studies have been performed worldwide, and contrasting results have been reported, including an absolute absence in fresh vegetables (Noto *et al*., 2018). Mottola *et al.* (2016) analyzed pre-cut lettuce, spinach, and arugula samples from Italy and obtained higher prevalences than the ones found in Costa Rica (20, 3.24 and 3.27% respectively). *A. butzleri* and *A. cryaerophilus* were the pathogenic species that were reported, the former being the predominant. Also, a study by Vicente-Martins *et al*. (2018) in Portugal revealed an even higher prevalence in vegetables ready for consumption in that country; however, no differentiation of results is made between different types of vegetables; the prevalence reported for these foods was 47.6%, from which 66.7% corresponded to isolates of *A. butzle*ri (Vicente-Martins *et al*., 2018). Recently, Mottola *et al.* (2021) reported a 14.5% prevalence (n=110) in ready-to-eat vegetables from southern Italy, being 93.8% *A. butzleri*.

A. butzleri is considered an emerging foodborne pathogen that has been isolated along the production chain, with the ingestion of contaminated food and water being its most likely transmission route to be acquired by humans. This species is the one that has been most frequently isolated in humans and in a greater variety of food products (Ferreira *et al.*, 2019). *A. butzleri* has been more investigated compared to other members of the genus due to its greater association with the production of pathologies. Some researchers claim that this species is the fourth most frequently detected *Campylobacter*-like organism in diarrheal samples from humans (Vicente-Martins *et al.*, 2018; Ferrei*ra et al.*, 2019).

In vegetable samples, the few studies published indicate that *A. butzleri* is the most prevalent. In fact, until 2016, *A. butzleri* was the unique species isolated from this type of sample (Hausdorf *et al.,* 2013). In 2016, 4 *A. cryaerophilus* isolates were obtained by Mottola *et al.* (2016) from 37 samples of pre-cut spinach (ready-to-eat), and it was not until 2017 that Ramees *et al.* reported the isolation of *A. skirrowii* from carrot and beet roots.

It is important to highlight that Costa Rican national guidelines stipulate the absence/25 g of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* as microbiological criteria for fresh-cut and packed vegetables. Based on the above, it is important to emphasize that *Aliarcobacter* spp. is not included as a parameter to be analyzed. In addition, these guidelines label this category of food as type B, which means that due to their nature, composition, process, handling, and target population, they carry a medium probability of causing damage to health (MAG-MEIC, 2018).

Virulence genes are denominated as putative due to the amino acid structures and functions corresponding to other genomic structures that have already been characterized (Rivera Palomino, 2015). The presence and distribution of putative virulence genes in *Aliarcobacter* recovered from vegetables highlighted a complex virulence profile, suggesting that many isolates are potential sources of illness. Of the *Aliarcobacter* species isolated from fresh vegetables, the genes *pldA, mviN* and *tly* were the most frequent ones, and *irgA, mviN, cj1349, tlyA* and *cadF* were the most frequent in ready-to-eat vegetables. Similar results have been reported by several researchers, including Collado *et al*. (2014), Karadas *et al.* (2013), Mottola *et al.* (2016), and Zacharow *et al.* (2015). The detection of *mviN* and *tlyA* genes may be associated with host cell invasion since *mviN* traduces the *mviN* protein and is related to peptidoglycan synthesis in the host's cell, helping to create cellular lysis, and *tlyA* generates a pore in the host's cellular membrane, facilitating lysis (Girbau *et al*., 2015).

This is the first report of the detection of the *Aliarcobacter* genus from vegetable sources usually consumed fresh in salads from Costa Rica, as well as the first report of the pathogenic species *A. butzleri* and *A. skirrowii* having virulence factors that might represent a risk for consumers. Future research should be performed to devise control strategies for these bacteria at both industrial and household levels. Also, more studies are needed to understand the potential relationship between the prevalence of the putative virulence genes and the pathogenic properties of the *Aliarcobacter* isolated.

References

Araya-Quesada Y, Jiménez-Robles A, Ivankovich-Guillén C, García-Barquero ME, 2014. Hábitos de consumo de embutidos en el cantón de San Carlos y el área metropolitana de Costa Rica. Tecnología en Marcha 27:113-24. [Article in Spanish].

Barboza K, Cubillo Z, Castro E, Redondo-Solano M, Fernández-Jaramillo H, Arias-Echandi ML, 2017. First isolation report of Arcobacter cryaerophilus from a human diarrhea sample in Costa Rica. Rev Inst Med Trop Sao Paulo 59:e72.

Calvo G, Arias ML, Fernández H, 2013. Arcobacter: un patógeno emergente de origen alimentario. ALN 63:164-72. [Article in Spanish].

Collado L, Figueras MJ, 2011. Taxonomy, epidemiology, and clinical relevance of the genus Arcobacter. Clin Microbiol Rev 24:174-92.

Collado L, Jara R, Vásquez N, Telsaint C, 2014. Antimicrobial resistance and virulence genes of Arcobacter isolates recovered from edible bivalve molluscs. Food Control 46:508-12.

D'sa EM, Harrison MA, 2005. Effect of pH, NaCl content, and temperature on growth and survival of Arcobacter spp. J Food Prot 68:18-25.

Douidah L, de Zutter L, Baré J, De Vos P, Vandamme P,

Vandenberg O, Vander Abeele A, Houf K, 2012. Occurrence of putative virulence genes in Arcobacter species isolated from human and animal. J Clin Microbiol 50:735-41.

Ferreira S, Oleastro M, Domingues F, 2019. Current insights on Arcobacter butzleri in food chain. Curr Opin Food Sci 26:9-17.

Ferreira S, Queiroz JA, Oleastro M, Domingues FC, 2016. Insights in the pathogenesis and resistance of Arcobacter: a review. Crit Rev Microbiol 43:364-83.

Girbau C, Guerra C, Martínez-Malaxetxebarria I, Alonso R, Fernández-Astorga A, 2015. Prevalence of ten putative virulence genes in the emerging foodborne pathogen Arcobacter isolated from food products. Food Microbiol 52:146-9.

Harmon KM, Wesley IV, 1996. Identificatin of Arcobacter isolates by PCR. Lett Appl Microbiol 23:241-4.

Hausdorf L, Fröhling A, Schlüter O, Klocke M, 2011. Analysis of the bacterial community within carrot wash water. Can J Microbiol 57:447-52.

Hausdorf L, Neumann M, Bergmann I, Sobiella K, Mundt K, Fröhling A, Schlüter O, Klocke M, 2013. Occurrence and genetic diversity of Arcobacter spp. in a spinach-processing plant and evaluation of two Arcobacter-specific quantitative PCR assays. Syst Appl Microbiol 36:235-43.

Karadas G, Sharbati S, Hänel I, Messelhäußer U, Glocker E, Alter T, Gölz G, 2013. Presence of virulence genes, adhesion and invasion of Arcobacter butzleri. J Appl Microbiol 115:583-90.

Lake IR, Barker GC, 2018. Climate change, foodborne pathogens and illness in higher-income countries. Curr Envir Health Rpt 5:187-96.

MAG-MEIC, 2018. Decreto ejecutivo No. 41420-C2018. Publica Resolución N° 402-2018 (COMIECO-LXXXIII) de fecha 28/06/2018 y su Anexo: "Reglamento Técnico Centroamericano RTCA 67.04.50:17 Alimentos. Criterios Microbiológicos para la Inocuidad de los Alimentos". In: Official Journal, 238, 21/12/2018. [Decree in Spanish].

Molva C, Atabay HI, 2016. Prevalence and diversity of Arcobacter spp. in retail chicken meat in Turkey. Microbiol Res 7:6578.

Mottola A, Bonerba E, Bozzo G, Marchetti P, Celano GV, Colao V, Terio V, Tantillo G, Figueras MJ, Di Pinto A, 2016. Occurrence of emerging food-borne pathogenic Arcobacter *s*pp. isolated from pre-cut (ready-to-eat) vegetables. Int J Food Microbiol 236:33-7.

Mottola A, Ciccarese G, Sinisi C, Savarino AE, Marchetti P, Terio V, Tantillo G, Barrasso R, Di Pinto A, 2021. Occurrence and characterization of Arcobacter spp. from ready-to-eat vegetables produced in Southern Italy. Ital J Food Saf 10:8585.

Neill SD, Ellis WA, O'Brien JJ, 1979. Designation of aerotolerant Campylobacter-like organisms from porcine and bovine abortions to the genus Campylobacter. Res Vet Sci 27:180-6.

Nieva-Echavarría B, Martinez-Malaxetxebarria I, Girbau C, Alonso R, Fernández-Astorga A, 2013. Prevalence and genetic diversity of Arcobacter in food products in the north of Spain. J Food Prot 76:1447-50.

Noto AM, Sciortino S, Cardamone C, Ciravolo C, Napoli C, Alio V, Arculeo P, Oliveri G, Costa A, 2018. Detection of Arcobacter spp. in food products collected from Sicilia region: a preliminary study. Ital J Food Saf 7:7171.

Perez-Cataluña A, Salas-Masso N, Diéguez AL, Balboa S, Lema A, Romalde JL, Figueras MJ, 2018. Revisiting the taxonomy of the genus Arcobacter: getting order from the chaos. Front Microbiol 9:2077

Pérez-Cataluña A, Salas-Massó N, Diéguez AL, Balboa S, Lema L, Romalde JL, Figueras MJ, 2019. Corrigendum (2): revisiting the taxonomy of the genus Arcobacter: getting order from the chaos. Front Microbiol 10:2253.

Ramees TP, Dhama K, Karthik K, Rathore RS, Kumar A, Saminathan M, Tiwari R, Malik YS, Singh RK, 2017. Arcobacter: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - a comprehensive review. Vet Q 37:136-61.

Rivera Palomino GO, 2015. Determinación de la presencia de los genes putativos de virulencia de Arcobacter en diferentes medios de crecimiento, temperaturas y condiciones atmosféricas. Available from: https://bdigital.zamorano.edu/server/api/core/bitstreams/18ae2777-e9a8-4eff-aab6-ef143a17197a/content. [Material in Spanish].

Shah AH, Saleha AA, Zunita Z, Murugaiyah M. 2011. Arcobacter - an emerging threat to animals and animal origin food products? Trends Food Sci Technol 22:225-36.

Simaluiza RJ, Ambuludi DR, Fernández H, 2021. First case of diarrhea due to Aliarcobacter butzleri (formerly Arcobacter butzleri) in Ecuador. Infect Dis Now 51:564-6.

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

Vicente-Martins S, Oleastro M, Domingues FC, Ferreira S, 2018. Arcobacter spp. at retail food from Portugal: prevalence, genotyping and antibiotics resistance. Food Control 85:107-12.

Winters DK, Slavik MF, 2000. Multiplex PCR detection of Campylobacter jejuni and Arcobacter butzleri in food products. Molec Cell Probes 14:95-9.

Zacharow I, Bystroń J, Wałecka-Zacharska E, Podkowik M, Bania J, 2015. Genetic diversity and incidence of virulence-associated genes of Arcobacter butzleri and Arcobacter cryaerophilus isolates from pork, beef, and chicken meat in Poland. Biomed Res Int 2015:956507.