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Research Paper

Arcobacter butzleri and *A. cryaerophilus* in human, animals and food sources, in southern Chile

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Abstract

The isolation frequency of *Arcobacter* species in children with diarrhea, fowls, mammals and food of avian and marine origin was established. In all the samples it was possible to isolate *Arcobacter* species corresponding 201 (39.4%) to *A. butzleri* and 24 (4.7) to *A. cryaerophilus*. Both species were simultaneously isolated in 19 (3.7%) being *A. butzleri* the most frequently isolated species.

Key words: Arcobacter diarrhea, reservoirs, food.

Introduction

Arcobacter sp. belongs to Campylobactereaceae family as was proposed by Vandamme *et al.* (1991). This genus included 18 species with *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* been considered the only emerging enteropathogens of relevance for animals and humans (Lehner *et al.*, 2005; Wesley and Miller, 2010; Collado and Figueras, 2011; Euzéby, 2014). *A. skirrowii* has been isolated from sheep and cattle with diarrhea, aborted pig fetus, and chronic and acute diarrhea in humans. *A. cryaerophilus* and *A.butzleri* have been associated with abortion and enteritis in animals as well as with diarrhea and bacteremia in children and adults. The transmission of these organisms occurred by fecal-oral, waterborne, by food of animal origin or by direct contact with animals (Lehner *et al.*, 2005; Wesley and Miller, 2010; Collado and Figueras, 2011).

In Chile there is scarce information regarding this group of bacteria. Fernández *et al.* (2004) reported the first isolates in this country. Due to the clinical relevance of *Arcobacter* as foodborne pathogen and the need to get more information related to its ecological distribution and prevalence, we present this communication. We are reporting the prevalence of *Arcobacter* in children with diarrhea, in animal reservoirs and in food of animal origin. The study was performed in the south part of Chile (Los Rios Region, 39º16' and 40º41' latitude South and 71º35' longitude West until the Pacific Ocean).

Material and Methods

We studied a total of 510 samples. The type and origin of the samples are described in Table 1. Human feces (approx. 2 g) were inoculated into *Arcobacter* broth (20 mL) and food samples (approx. 25 g previously vortex) were added into 125 mL of *Arcobacter* broth (Houf *et al.*, 2001). After 48 h of incubation at 30 °C under aerobic conditions, 100 microliters aliquots were streaked onto *Arcobacter* agar plates (Houf *et al.*, 2001). Additionally, 400 microliters of enrichment broth were filtrated onto blood agar plates using a 0.45 mμ filter (Fernández *et al.*, 2004). All the plates were incubated under the same conditions described above. Colonies with 2 to 4 mm diameter and smooth borders were tested for Gram stain, wet preparation for contrast microscopy, and oxidase and catalase tests. Those colonies with oxidase and catalase positive of rods gram negative with characteristic motility spinning around their long axes under the contrast microscope were considered presumptively *Arcobacter* sp.. Those colonies were streaked in blood agar plates for confirmatory testing using multiplex PCR as proposed by Houf *et al.* (2000) for the confirmation of the three main species of *Arcobacter*.

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Table 1 - Prevalence of *Arcobacter* species among human, animal and food samples.

Origin	Type	Number	*A. butzleri*		*A. cryaerophilus*		*A. butzleri* + *A. cryaerophilus*	
			N°	%	N°	%	N°	%
Children	Feces	83	3	3.6	0	0	0	0
Pig	Feces	135	55	40.7	13	9.6	12	8.9
Bovine	Feces	75	20	26.7	5	6.7	3	4.0
Chicken	Feces	20	2	10.7	4	20.0	0	0
Chicken	Meat	125	90	72.0	2	1.6	3	2.4
Chicken	Stomach	25	8	32.0	0	0	0	0
Chicken	Liver	25	18	72.0	0	0	1	4.0
Seafood	Mussels	22	5	22.7	0	0	0	0
Total		510	201	39.4	24	4.7	19	3.7

Results

As indicated on Table 1, *Arcobacter* was isolated from all the sample types. A total of 201 (39.4%) strains of *A. butzleri*, and 24 (4.7%) of *A. cryaerophilus* were isolated. In 19 (3.7%) of the samples studied both species were recovered simultaneously.

From children with diarrhea and from mussels, only *A. butzleri* was isolated (3.6% and 22.7%, respectively).

In chicken feces, *A. cryaerophilus* was most frequently isolated (20%) than *A. butzleri* (10%) but in pig and bovine feces occurred the opposite. The frequency of *A. butzleri* was 40.7% in pigs and 26.7% in bovines while for *A. cryaerophylus* they were 9.6 and 6.7%, respectively.

High frequencies of *Arcobacter* were found in poultry meat ready for human consumption (chicken meat 92%, muscular stomach 32%, and liver 92%).

Discussion

A. butzleri has been described as the *Arcobacter* species more frequently isolated from clinical and environmental specimens, as well as from food of animal origin (Lehner *et al.*, 2005; Wesley and Miller, 2010; Collado and Figueras, 2011), and it was confirmed as the most prevalent specie in this study. *A. butzleri* was the only *Arcobacter* specie isolated from children with diarrhea with 3.6% prevalence. The prevalence of *Arcobacter* in this study was higher to the prevalence found by Prouzet-Mauleon *et al.* (2006) in France (1%) and to the prevalence previously reported by Collado *et al.* (2013) in Valdivia, Chile (1.7%). However, the prevalence of *Arcobacter* in this study was similar to the prevalence observed by Vandenberg *et al.* (2004) in Belgium (3.5%). Similar to the reports of Prouzet-Mauleon *et al.* (2006) and Collado *et al.* (2013), we were unable to recovery *A. skirrowii* or *A. cryaerophilus* from feces of children with diarrhea. The only study in which *A. cryaerophilus* was isolated from feces of children with diarrhea was the report of Vandenberg *et al.* (2004) but as in other studies included the present one, *A. skirrowii* was not recovered.

In Europe, the reports indicated that *Arcobacter* species represent the 4th place in frequency of isolation among the member of the Campylobactereaceae family in samples of fecal origin (Vandenberg *et al.*, 2004; Prouzet-Mauleon *et al.*, 2006). The high prevalence of *A. butzleri* found in diarrhea, similarly at is occurs with *Campylobacter jejuni*/*coli*, might be an indicator of similar risk factors in the environment (Wesley and Miller, 2010, Fernández, 2011).

In chicken feces, *A. butzleri* was isolated (10%) as well as *A. cryaerophilus* (20%). The prevalence found in this study is similar to previous studies (Fernández *et al.*, 2007; Houf, 2010). However, the prevalence of *Arcobacter* species in pig and bovine feces was lower that the prevalence of *Campylobacter* species in the same type of samples (Table 1). The findings in this study were similar to results previously reported in the bovine and pig samples (Fernández, 2011; Fernández *et al.*, 2007; Houf, 2010).

Some investigators have proposed that the low prevalence of *Arcobacter* species in chickens is an indicator that this organism is not a natural intestinal commensal in chicken and represent only a transient organism incapable to colonize the gut chicken. A potential explanation for this phenomenon is that the normal corporal temperature in chicken (40.5 to 42 °C) represents a limiting factor for the colonization of chicken with *Arcobacter* species because their optimal growth temperature range is from 26 to 30°C, contrasting with *Campylobacter* that is recovered in high prevalence in chicken due to its optimal growth temperature that is around 42°C (Wesley and Miller, 2010; Houf, 2010). However, in contrast from the animal samples, the recovery of *Arcobacter* from poultry meat ready for human consumption is high (92% in chicken meat, 32% in muscular stomach, and 92% in liver). These results of prevalence of *Arcobacter* in poultry meat are in agreement with those previously reported (Fernández *et al.*, 2001; Collado and Figueras, 2011). According to Wesley and Miller (2010)

and Houf (2010), the explanation for this discrepancy is that poultry may be contaminated in processing plants and the temperature used for storage of the product (4°C and/or room temperature) may favor the colonization by external sources.

The frequency of recovery *Arcobacter* species was higher in feces from bovine, pigs, and poultry meat samples. Among those samples, *A. butzleri* and *A. cryaerophilus* were recovered simultaneously. This observation has been previously reported (Lehner *et al.*, 2005; Collado and Figueras, 2011; Houf, 2010).

The prevalence of *A. butzleri* in mussels was 22.7%, this prevalence was different from previous reports by Fernandez *et al.* (2001) and Collado *et al.* (2009) who reported higher prevalence of *A. butzleri* and the recovery of additional *Arcobacter* species in the same type of seafood.

In summary, this report confirmed the presence of *Arcobacter* species in children with diarrhea in the south region of Chile. In addition, this report confirmed the presence of *Arcobacter* in animal reservoirs and in food of animal origin such as poultry meat and seafood. We believe that the presence of *Arcobacter* may be an indicator of fecal contamination in the human environment and food supplies. Therefore, it is imperative to establish a constant survey to determine its environmental distribution, its presence in other animal sources as well as the epidemiological relationship between *Arcobacter* strains isolated from different sources and their mode of transmission. This information will contribute to clarify and a better understanding of the epidemiology of this emerging enteropathogen.

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