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Research Note

Prevalence of *Arcobacter* in Meat and Shellfish

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ABSTRACT

Arcobacter is considered an emergent foodborne and waterborne enteropathogen. However, its prevalence in foods of animal origin is only partially known, because most studies have been concentrated on poultry, pork, and beef, and methods applied do not allow identification of all currently accepted *Arcobacter* species. We investigated the prevalence of *Arcobacter* in 203 food samples, 119 samples of seven different types of meats and 84 samples of four types of shellfish. Isolates were identified in parallel by using a published multiplex PCR method and a recently described 16S rDNA restriction fragment length polymorphism method that allows all currently accepted *Arcobacter* species to be characterized. The global prevalence of *Arcobacter* was 32%; it was highest in clams (5 of 5 samples, 100%) and chicken (9 of 14 samples, 64.3%) followed by pork (9 of 17 samples, 53.0%), mussels (23 of 56 samples, 41.1%), and duck meat (2 of 5 samples, 40.0%). Turkey meat and beef had a similar recovery rate (10 of 30 samples, 33.3%; 5 of 16 samples, 31.3%; respectively), and rabbit meat had the lowest rate (1 of 10 samples, 10.0%). No arcobacters were found in oysters, frozen shrimps, or sausages. This food survey is the first in which five of the seven accepted *Arcobacter* species have been isolated. *Arcobacter butzleri* was the most prevalent species (63.0% of isolates) followed by *Arcobacter cryaerophilus* (26.6%), *Arcobacter mytili* (4.7%), *Arcobacter skirrowii* (3.1%), and *Arcobacter nitrofigilis* (3.1%). Three (4.7%) of the isolates were classified as belonging to three potentially new phylogenetic lines. Our results indicated that *Arcobacter* species are widely distributed in the food products studied.

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The genus *Arcobacter* (previously known as a group of aerotolerant campylobacters) has gained increased attention as an emergent waterborne and foodborne enteropathogen *(2, 12)*. Currently, the genus contains seven species: *Arcobacter butzleri, Arcobacter cryaerophilus* (with two DNA groups, 1A and 1B), *Arcobacter skirrowii, Arcobacter nitrofigilis (34), Arcobacter cibarius (14), Arcobacter halophilus (7),* and *Arcobacter mytili (3). A. butzleri, A. cryaerophilus,* and *A. skirrowii* have been associated with gastrointestinal disease and bacteremia in humans and with abortion and diarrhea in animals *(12)*. Nevertheless, information on their pathogenicity and virulence factors is limited *(12). A. butzleri* is the most commonly isolated species and has been classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods *(16). Arcobacter* species have been isolated frequently from drinking water reservoirs *(17)* and recently from fecally contaminated bathing waters *(4)*. These bacteria also are present in feces of livestock *(35)* and in poultry, beef, and pork meats *(2, 20, 32)*; however, few studies have been conducted with other types of meats. Despite the ability of molluscs to concentrate bacterial pathogens, the prevalence of *Arcobacter* in shellfish has rarely been studied *(8, 23)*.

In previous studies, the molecular method used *(15)* allowed the identification of only three of the seven species included in the genus: *A. butzleri, A. cryaerophilus,* and *A. skirrowii.* Recently, we have published a fast molecular method, a 16S rDNA restriction fragment length polymorphism (RFLP) assay, that enables the identification of all the currently accepted *Arcobacter* species *(9),* including the new species *A. mytili (3).* The main objective of this study was to use this identification method to investigate the prevalence and diversity of the *Arcobacter* species in different meat and shellfish samples to establish the true importance of these emergent enteropathogens in common foods. The results of the 16S rDNA–RFLP assay were compared with those obtained with the commonly used multiplex PCR (m-PCR) method of Houf et al. *(15)*.

MATERIALS AND METHODS

Samples and isolation procedure. A total of 203 samples were studied: 119 meat samples from retail markets in Catalonia (northeastern Spain) obtained between June 2006 and March 2008 (14 chicken, 30 turkey, 5 duck, 17 pork, 16 beef, 10 rabbit, and 27 sausage) and 84 shellfish samples (56 mussel, 5 clam, 6 oyster, and 17 frozen shrimp). Shellfish samples also were obtained from local markets, with the exception of the mussels, which were harvested from a mussel farm at the Ebro River delta.

Within 24 to 48 h of collection, 10-g samples of the meat or shellfish were each homogenized with 90 ml of *Arcobacter* enrichment broth supplemented with cefoperazone, amphotericin B, and teicoplanin (Oxoid, Basingstoke, UK) in tightly sealed stomacher bags. After incubation for 48 h at 30°C under aerobic conditions, 200 μl of the broth was inoculated onto blood agar plates following the procedure described by Collado et al. *(4)*. Four

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TABLE 1. *Prevalence and diversity of* Arcobacter *species from foods*[a]

Sample type	No. of samples	No. (%) of samples positive for:							
		Any *Arcobacter*	*A. butzleri*	*A. cryaerophilus*	*A. mytili*	*A. skirrowii*	*A. nitrofigilis*	*A. skirrowii* like[b]	Other arcobacters
Mussels	56	23 (41.1)[c]	10 (43.5)	8 (35.0)	3 (13.0)		2 (9.0)		1 (4.3)
Oysters	6								
Clams	5	5 (100)[d]	2 (40.0)	4 (80.0)		1 (20.0)			
Shrimp	17								
Chicken meat	14	9 (64.3)	9 (100)						
Turkey meat	30	10 (33.3)	8 (80.0)	2 (20.0)					
Duck meat	5	2 (40.0)	1 (50.0)	1 (50.0)					
Pork	17	9 (53.0)	6 (66.7)			1 (11.1)		1 (11.1)	1 (11.1)
Rabbit meat	10	1 (10.0)	1 (100)						
Beef	16	5 (31.3)	3 (60.0)	2 (40.0)					
Sausage	27								
Total	203	64 (32.0)	40 (62.5)	17 (26.6)	3 (4.7)	2 (3.1)	2 (3.1)	1 (1.6)	2 (3.1)

[a] Identification by 16S rDNA–RFLP assay.
[b] A possible new species suggested by On et al. *(27)*.
[c] In one sample, both *A. butzleri* and *A. cryaerophilus* were present.
[d] In one sample, *A. butzleri, A. cryaerophilus,* and *A. skirrowii* were present.

small colorless or beige to off-white translucent colonies were selected from each plate and transferred to blood agar to obtain pure cultures.

Detection and identification of *Arcobacter*. For the detection of *Arcobacter,* 400 μl of the incubated enrichment broth was used for the m-PCR using primers and conditions described by Houf et al. *(15)* for detection and identification of *A. butzleri, A. cryaerophilus,* and *A. skirrowii.* In parallel, colonies were submitted to Gram staining and oxidase and motility tests. Final identification of the colonies was made using the m-PCR *(15)* and a recently described 16S rDNA–RFLP *Arcobacter* identification method, which consists of amplification of 1,026 bp of the 16S rRNA gene and digestion with the *Mse*I endonuclease *(9).* When the four isolates on each plate were identified as the same species, only one was recorded for the analysis of prevalence. When the identification methods gave different results, the 16S rRNA gene was sequenced to establish correct identification using the universal primers developed by Martinez-Murcia et al. *(22).* The generated sequences were then compared with those deposited in GenBank using BlastN (NCBI, Bethesda, MD), and a phylogenetic tree was constructed using the MEGA4 software (Sudhir Kumar, Center for Evolutionary Functional Genomics, Tempe, AZ).

Reference strains. The type strains of *A. butzleri* (LMG 10828), *A. cryaerophilus* (LMG 9904), *A. skirrowii* (LMG 6621), *A. halophilus* (LA31B), *A. cibarius* (CECT 7203), *A. nitrofigilis* (CECT 7204), and *A. mytili* (CECT 7386) were used as controls.

Statistical analyses. Fisher's exact test was used to compare the prevalence of *Arcobacter* in meat and shellfish samples. Statistical analyses were performed using the Statistical Package for Social Sciences (v. 15.0, SPSS Inc., Chicago, IL). Statistical significance was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

The results on the prevalence and diversity of the *Arcobacter* species are summarized in Table 1. *Arcobacter* spp. were present in 64 (32.0%) of the samples; they were most prevalent in clams (5 of 5 samples, 100%) and chicken (9 of 14 samples, 64.3%) followed by pork (9 of 17 samples, 53.0%), mussels (23 of 56 samples, 41.1%), and duck meat (2 of 5 samples, 40.0%). Turkey meat and beef had similar prevalences (10 of 30 samples, 33.3%; 5 of 16 samples, 31.3%; respectively), and rabbit meat had the lowest prevalence (1 of 10 samples, 10.0%). In oysters, frozen shrimp, and sausages, no arcobacters were recovered or detected. No significant differences were found between meat and shellfish samples ($P = 0.38$). Although 59 of the 64 culture-positive samples were positive by m-PCR assay (from enrichment broth cultures), the other five (7.8%) were negative by m-PCR, possibly because of PCR inhibition caused by coarse particles and solidified fat, as previously described *(15).*

Discrepancies between the identification methods. The 16S rDNA–RFLP assay and the m-PCR method produced the same identity results for 59 of 67 strains (88.1% agreement). Of the eight isolates that yielded dissimilar results, five were identified as *A. skirrowii* and three were identified as *A. cryaerophilus* based on the m-PCR results. However, of those five *A. skirrowii* isolates, two had the characteristic RFLP pattern of *A. nitrofigilis* and three had the pattern of the recently proposed species *A. mytili (3).* This discrepancy occurred because when the m-PCR method was designed, the strain of *A. nitrofigilis* tested did not produce any amplification *(15),* and at that time *A. mytili* was unknown. Of the three strains that were identified as *A. cryaerophilus* by m-PCR assay, two strains (F4 and F41), isolated from mussels and pork samples, respectively, were assigned to two potentially new phylogenetic lines on the basis of their 16S rDNA–RFLP patterns and 16S rRNA gene sequences. The phylogenetic tree derived from this gene shows these strains as independent branches (Fig. 1). The remaining strain (F61, from pork) had a restriction pattern identical to that of *A. butzleri,* but the analysis of its 16S rRNA gene sequence (Fig. 1) indicated a 99.8% sim-

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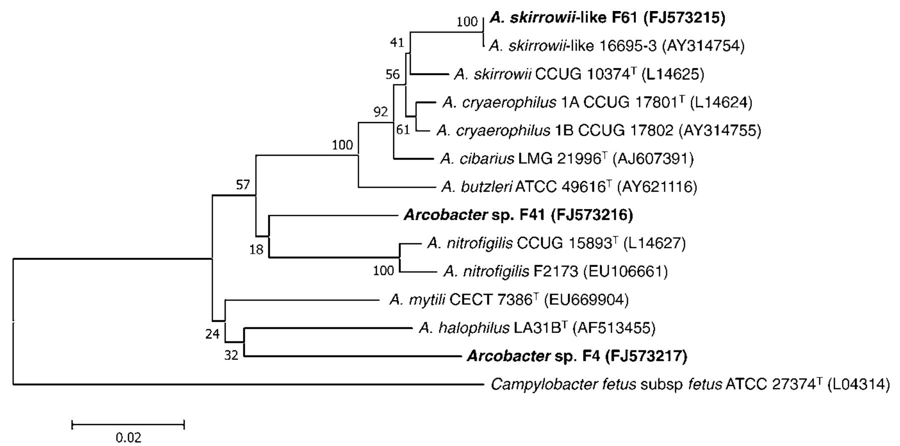

FIGURE 1. *Neighbor-joining phylogenetic tree showing the relationship between the three potentially novel species and the other members of the genus* Arcobacter *based on 1,409 nucleotides from the 16S rRNA gene. The stability of the groupings was estimated by bootstrap analysis (1,000 replications).*

ilarity with a potential new *Arcobacter* species recovered from porcine abortion material in Denmark *(27)*. These data agree with those from other studies in which some isolates could not be identified to species *(31, 32)*, indicating a diversity of potentially new *Arcobacter* species in foods. The misidentification problems that occurred here with the m-PCR method *(15)* were previously discussed when the 16S rDNA–RFLP identification method was proposed *(9)*. The application of the 16S rDNA–RFLP method in this work, apart from being a reliable identifier of all known *Arcobacter* spp., has been very useful for recognizing potential new species since new RFLP patterns have been obtained.

Prevalence of *Arcobacter* species. *A. butzleri* was the most prevalent species in this study (40 of 64 samples, 62.5%), followed by *A. cryaerophilus* (17 of 64 samples, 26.6%), *A. mytili* (3 of 64 samples, 4.7%), and *A. skirrowii* and *A. nitrofigilis* (the same prevalence: 2 of 64 samples, 3.1%). *A. skirrowii, A. butzleri,* and *A. cryaerophilus* were detected in one sample of clams, and *A. butzleri* and *A. cryaerophilus* were detected in one mussel sample (Table 1). *A. butzleri* was the most common species recovered from most types of meats and shellfish, except in clams, where *A. cryaerophilus* prevailed. *A. butzleri* is an emergent foodborne pathogen recently classified as a serious hazard to human health *(16)*, and high prevalence was found in all studies listed in Table 2. The 16S rDNA–RFLP identification method used here enabled the differentiation of the two DNA groups (1A and 1B) defined for *A. cryaerophilus.* Only one strain recovered from turkey meat belonged to group 1A, and the rest belonged to group 1B. This tendency to recover group 1B more frequently was reported in a study of broiler chicken carcasses *(33)*.

In the present study, the new species *A. mytili* was recovered only from mussels, although in general it had a prevalence similar to that of *A. skirrowii* and *A. nitrofigilis.* The three isolates of this new species were recovered from three different mussel samples obtained on two different sampling dates. However, the *A. mytili* strains belonged to only two different genotypes, as determined by an enterobacterial repetitive intergenic consensus PCR assay *(3)*. The true prevalence of *A. mytili* and *A. nitrofigilis* probably is being underestimated because the commonly used m-PCR method *(15)* wrongly identifies these species as *A. skirrowii.*

The infrequent recovery of *A. skirrowii* (4.7%) in this study is in agreement with the results of previous studies of meat samples *(13, 19)*.

The only two *A. nitrofigilis* isolates recovered in the present study were from mussels and were identified on the basis of the 16S rDNA–RFLP assay, 16S rRNA gene sequencing (GenBank accession nos. EU106661 and EU106662), and urease activity, which is characteristic of this species *(24)*. Originally, *A. nitrofigilis* was isolated from the roots of the salt marsh plant *Spartina alterniflora,* which grows in brackish environments *(24)*. The Ebro River delta is a brackish environment, so it is not surprising that we isolated this species from the mussels farmed in this area. To our knowledge, since the description of *A. nitrofigilis* by McClung et al. in 1983 *(24)* only one group of researchers has isolated and phenotypically identified this species *(23)*. From that study, it was not possible to determine the prevalence or the number of isolates or whether the isolates were recovered from mussels or water. The present study is the first since 1983 that has genetically identified *A. nitrofigilis.*

Prevalence of *Arcobacter* in different types of meats. Several factors, i.e., different geographical regions, hygienic conditions, and isolation methods, can affect comparison of our results with those of previous studies. However, the prevalence of *Arcobacter* in chicken meat (64.3%) is consistent with prevalences found in previous studies, which ranged from 20 to 73% (Table 2). However, when chicken skin and carcass samples were analyzed, the prevalence was 100% *(2)*. The results reported by Zanetti et al. *(39)* were not considered in Table 2 because *Arcobacter* was not recovered from sausages, poultry, or turkey meats and only one beef sample was positive for *Arcobacter.* This result probably is linked to the inappropriate isolation protocols used in that study. The present study is the first in which the prevalence of *Arcobacter* in duck and turkey meats (40 and 33.3%, respectively) has been evaluated. The only previous studies of *Arcobacter* prevalence in these products have been conducted with duck carcasses (80%) *(29)* and turkey carcasses (93%) *(1)* or mixtures of turkey skeletal

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TABLE 2. *Reported prevalence of* Arcobacter *in meats and shellfish*[a]

Sample type	No. of samples studied	% positive samples	Identification method	Country	Reference
Chicken	50	38.0	Biochemical identification	Thailand	*37*
	94	62.0	m-PCR *(15)*	Ireland	*32*
	100	23.0	m-PCR *(18)*	Japan	*19*
	51	59.0	m-PCR *(18)*	Japan, Thailand	*25*
	22	73.0	m-PCR *(15)*	Australia	*30*
	15	20.0	PCR *(11)*	United States	*36*
	52	65.4	m-PCR *(15)*	Belgium	*13*
	14	50.0	m-PCR *(15)*	Belgium	*15*
	220	24.1	Biochemical identification	The Netherlands	*6*
Pork	50	16.0	Biochemical identification	Thailand	*37*
	101	35.0	m-PCR *(15)*	Ireland	*32*
	100	7.0	m-PCR *(18)*	Japan	*19*
	21	29.0	m-PCR *(15)*	Australia	*30*
	45	51.1	PCR *(11)*	United States	*36*
	200	32.0	PCR *(11)*	United States	*26*
	194	0.5	Biochemical identification	The Netherlands	*6*
	299	55.8	DNA Probe *(38)*	United States	*5*
Beef	80	15.0	m-PCR *(15)*	Ireland	*10*
	50	28.0	Biochemical identification	Thailand	*37*
	108	34.3	m-PCR *(15)*	Ireland	*32*
	90	2.2	m-PCR *(18)*	Japan	*19*
	32	22.0	m-PCR *(15)*	Australia	*30*
	97	5.1	m-PCR *(15)*	Turkey	*28*
	45	28.9	PCR *(11)*	United States	*36*
	68	1.5	Biochemical identification	The Netherlands	*6*
Mussels	80	35.0	Biochemical identification	Chile	*8*
	NS	NS	Biochemical identification	Italy	*23*

[a] Only meat products (not carcasses, viscera, or skin) were considered. NS, not specified.

tissue and skin (77%) *(21).* In our study, *Arcobacter* prevalence in pork (53.0%) was higher than that found in other studies, which ranged from 7 to 35% (Table 2), and is similar to the 51.1 and 55.8% reported by Villarruel-López et al. *(36)* and Collins et al. *(5),* respectively.

In beef, the *Arcobacter* prevalence found in this study (31.3%) is consistent with the findings of other authors (Table 2). Our study is the first to find *A. butzleri* (10.0%) in rabbit meat. No arcobacters were detected in sausages, in agreement with results of a previous study *(39).* However, this finding is surprising considering the high prevalence of *Arcobacter* in pork (Table 2).

Prevalence of *Arcobacter* in shellfish. There are few reports of *Arcobacter* in shellfish, although these bacteria have been detected in oysters, as determined by analysis of 16S rDNA sequences *(31). Arcobacter* was not found in oysters in the present study, and although only five clam samples were investigated, all of them were positive for *Arcobacter.* This finding is important because all of the species recovered from this type of shellfish have been implicated in gastrointestinal diseases *(12). A. butzleri* and *A. nitrofigilis* have been recovered previously (prevalence not specified) from mussels and brackish water in Italy *(23).* In that study, the water quality of the samples associated with mussels was very poor, with high levels of fecal coliforms, confirming previous findings that most *Arcobacter* species enter the marine environment through sewage pollution *(3).* This pollution effect also may explain the presence of *Arcobacter* in other shellfish. In our study the prevalence of *Arcobacter* in mussels was high (23 of 56 samples, 41.1%), and four species and a potential new species were also recovered. A study conducted in Chile revealed a similar prevalence of *Arcobacter* in mussels (35%) *(8).*

This survey of several types of meat and shellfish revealed that about one-third of the samples were contaminated with several species of *Arcobacter.*

Note added in proof. After acceptance of this manuscript, *Arcobacter thereius* sp. nov. was described (Houf et al., *Int. J. Syst. Evol. Microbiol.,* in press), including strains termed *A. skirrowii*–like by On et al. *(27)*; therefore, the so referred strain recovered in our study (Table 1 and Fig. 1) belongs to this new species.

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