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Taxonomy, Epidemiology, and Clinical Relevance of the Genus *Arcobacter*

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INTRODUCTION

The genus *Arcobacter* has become increasingly important in recent years because its members have been considered emergent enteropathogens and potential zoonotic agents (64, 139). This genus is an atypical group within the epsilon subdivision of the proteobacteria because of its wide diversity of habitats and hosts (31, 162). Some *Arcobacter* species have been detected in or isolated from stools of patients with and without diarrhea and occasionally in association with bacteremia, endocarditis, and peritonitis (1, 64, 84, 95, 96, 100, 132, 167). In animals, arcobacters have been implicated in abortions, mastitis, and gastrointestinal disorders but have also been recovered from asymptomatic animals (152, 157). Despite that, the incidence of *Arcobacter* species is probably underestimated, due mainly to limitations in current detection and identification methods (153).

In recent years considerable progress has been made in understanding the taxonomy and pathogenicity of this group of microorganisms, and two reviews were provided independently by Ho et al. (64) and Snelling et al. (139) in 2006. Since then, important new contributions have been published, such as the complete genome of *Arcobacter butzleri* from a human clinical strain, which revealed detailed information about the physiology and genetics of this organism (110). This is the most important and prevalent species of the genus; it has been classified as a serious hazard to human health by the International Commission on Microbiological Specifications for Foods (81) and recently as a significant zoonotic pathogen (19). Taxonomic studies of this genus, as well as increased understanding of its routes of transmission and mechanisms of pathogenicity, justify a reevaluation (see, e.g., references 15, 24, 26, 65, 67, 69, 74, 94, 109, and 162). In the present overview, special emphasis is put on the information obtained from the genome sequence of *A. butzleri* and descriptions of novel *Arcobacter* species. Advances in the understanding of *Arcobacter* transmission routes are presented, as well as information from recent water and food surveys using novel detection, identification, and typing techniques.

TAXONOMY

The genus *Arcobacter* was proposed in 1991 by Vandamme et al. (149) to accommodate two aerotolerant *Campylobacter* species: *Campylobacter cryaerophila* (now *Arcobacter cryaerophilus*) and *Campylobacter nitrofigilis* (now *Arcobacter nitrofigilis*, the genus type species). The former was isolated from diverse origins (i.e., from the feces, reproductive tracts, and aborted fetuses of several farm animals and from the milk of cows with mastitis) (115). The latter species is a nitrogen-fixing bacterium

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isolated from the roots and root-associated sediments of *Spartina alterniflora*, a salt marsh plant (107). In 1992, Vandamme et al. (152) amended and enlarged the genus, with the reclassification of *Campylobacter butzleri* as *Arcobacter butzleri* and with the description of the new species *Arcobacter skirrowii*. *Arcobacter butzleri* was originally isolated from humans and animals with diarrhea (92), while *A. skirrowii* was obtained from the feces of lambs with diarrhea, aborted porcine, ovine, and bovine fetuses, and the prepuce of bulls.

Within the species *A. cryaerophilus*, two groups (named either 1A and 1B or 1 and 2) were defined based on different restriction fragment length polymorphisms (RFLP) of the 16S and 23S rRNA genes (93) and whole-cell protein and fatty acid contents (152). Also, using amplified fragment length polymorphism (AFLP) analysis, these groups were found to cluster separately (118). The *A. cryaerophilus* group 1B is much more prevalent than 1A (25, 78, 89, 133, 140), with both groups having so far been isolated simultaneously only from food products and from animal and human clinical samples (25, 89, 133, 140, 152). Some studies have indicated the need to clarify whether these two groups belong to two separate taxa (31, 148). Regarding this, the taxonomy of these two groups of *A. cryaerophilus* strains has recently been investigated using AFLP and the sequences of the *hsp60* gene (32), and the results suggested that the separation of the two groups should be abandoned and that the current type strain of this species (LMG 24291^{T}) should be exchanged for LMG 10829, which is more representative of the species (32).

Two additional species were described in 2005; one of them was *Arcobacter cibarius*, isolated from broiler carcasses in Belgium (75), and the other was *Arcobacter halophilus*, described on the basis of a unique strain recovered from a hypersaline lagoon in Hawaii (35). The latter represents the first obligate halophilic *Arcobacter* species. Very recently, six new species have been added to the genus, which now therefore includes 12 species. *Arcobacter mytili*, isolated from mussels and brackish water in Spain, was the first species of the genus that is unable to hydrolyze indoxyl acetate (24); *Arcobacter thereius* has been isolated from livers and kidneys of spontaneously aborted porcines and from duck cloacal samples (74); *Arcobacter marinus* (reported on the basis of only one strain) has been isolated from a mixed sample of seawater, starfish, and seaweeds in Korea (94); *Arcobacter trophiarum* was isolated from feces of fattening pigs in Belgium (34); *Arcobacter defluvii* was isolated from sewage samples (28); and *Arcobacter molluscorum* was recovered from mussels and oysters and is the second species of the genus that does not hydrolyze indoxyl acetate (52). One strain isolated from a chicken cloacal swab sample in Valdivia (Chile) showed 99.9% 16S rRNA gene similarity (GenBank accession number GU300768) with the sequence of the type strain of *A. trophiarum* (34), indicating that the strain, recovered from a different origin and region, belonged to this new species (M. J. Figueras, L. Collado, A. Levican, and H. Fernández, unpublished data).

An obligate microaerophilic organism that oxidizes sulfides was proposed as a potential new species, "*Candidatus* Arcobacter sulfidicus" (166), but a formal description does not yet exist. Additionally there are two potential new species recovered from mussels and from pork meat that are waiting to be formally described and named (25). The taxonomy of the genus *Arcobacter*, like those of other bacterial genera, has been based on the analysis of the 16S rRNA gene (163). In fact, from sequences deposited in public databases, the existence of several potentially new *Arcobacter* species can be inferred (110, 162). Recently published 16S rRNA gene phylogenetic analysis, constructed with nearly full-length 16S rRNA gene sequences of uncultured or not-yet-described species (>1,300-bp sequences deposited up to October 2009 at the MSU Ribosomal Database Project) (W. Miller personal communication) in combination with sequences of known *Arcobacter* spp., revealed that the new phylogenetic lines waiting to be described outnumber those already known (162). These potentially new *Arcobacter* species come from very different hosts and/or habitats, i.e., activated sludge and sewage, oil field environments, tidal and marine sediments, seawater, estuarine and river water, plankton, coral, tubeworms, snails, oysters, abalone, and associated with cod larviculture or with cyanobacterial mats (31, 44, 130, 136, 145, 162). Although most of them are sequences from uncultured bacteria, it is likely that several new species will be proposed in the near future. All these provide evidence that *Arcobacter* species inhabit very diverse environments, as indicated by Wesley and Miller (162).

Figure 1 shows the 16S rRNA gene phylogenetic relationships of the presently described species. The interspecies similarity among the 12 *Arcobacter* species included ranges from 92.1 to 98.9%. The higher value corresponds to the similarity of *A. cibarius* and *A. cryaerophilus* and the lower one to that of *A. thereius* and *A. halophilus*.

Some *Arcobacter* housekeeping genes, such as *gyrA* (2) and *rpoB-rpoC* (113), have been investigated to better differentiate the species and their phylogenetic relationships. However, in only a few recent studies, using the *rpoB* (24, 28, 52), *gyrB* (28, 52), and *hsp60* genes (28, 32, 34, 52), has the phylogeny of the genus been evaluated using all the type strains of the accepted species. The results from these genes were congruent with the 16S rRNA gene-based phylogeny (24, 28, 32, 34, 52), and they showed lower intra- and interspecies similarities and therefore a higher discriminatory power.

GENOMICS

Two *Arcobacter* genomes have already been sequenced to completion (110, 124). The first one obtained was *A. butzleri* (from the human strain RM4018, which is a derivative of the type strain), with 2.34 Mb and 2,259 coding sequences; 1,011 (45%) of the predicted proteins were assigned a specific function, 505 (22%) were attributed only a general function, and 743 (33%) were considered proteins of unknown function (110). A substantial proportion of the genome includes genes associated with the growth and survival of the bacteria under diverse environmental conditions, and there are pathways and loci associated with non-host-associated organisms (110). Also, putative virulence genes homologous to those described for *Campylobacter* (see below) were recognized by Miller et al. (110), which led those authors to indicate that *A. butzleri* can be considered a free-living, waterborne organism that might be rightfully classified as an emerging pathogen. Data from this genome showed an important number of genes involved in sulfur metabolism, which is typical of free-living taxa such as the unclassified *Epsilonproteobacteria Nitratiruptor* and *Sul-*

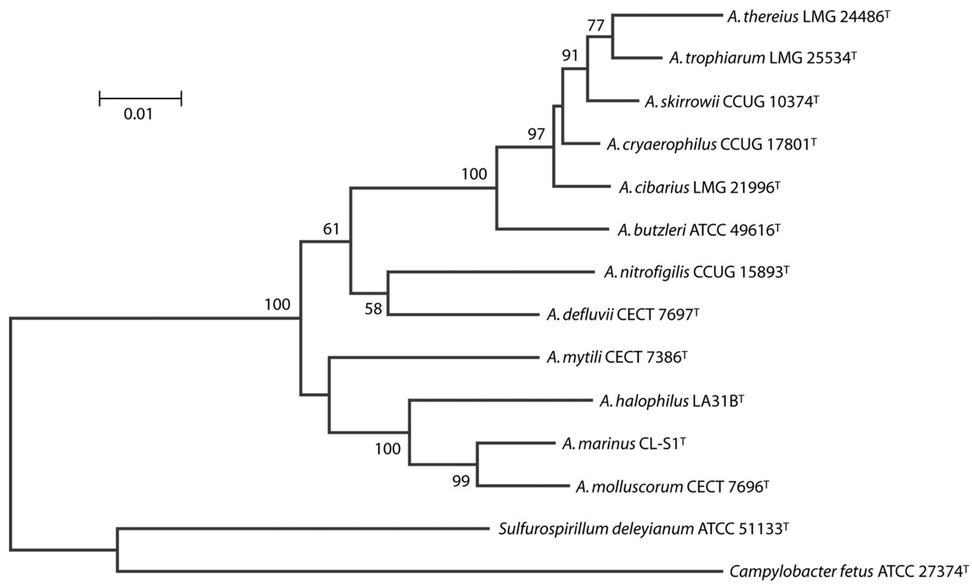

FIG. 1. Neighbor-joining phylogenetic tree showing the relationship of the described *Arcobacter* species on the basis of the 16S rRNA gene. Bootstrap values (>50%) based on 1,000 replications are shown at the nodes of the tree. Bar, 1 substitution per 100 nucleotides. ATCC, American Type Culture Collection, Manassas, VA; CECT, Colección Española de Cultivos Tipo, Universidad de Valencia, Valencia, Spain; CCUG, Culture Collection of the University Göteborg, Göteborg, Sweden; LMG, Culture Collection of the Laboratorium voor Microbiologie Gent, Universiteit Gent, Gent, Belgium. Equivalence culture collection numbers *A. halophilus* LA31B^T, ATCC BAA-1022^T; *A. marinus* CL-S1^T, CECT 7727^T.

furovum and also *Sulfurimonas*, a member of the *Helicobacteraceae* (110), which suggests that a reevaluation is needed to clarify whether the inclusion of *Arcobacter* in the family *Campylobacteraceae* is correct or not (110, 122). This need is also reinforced by the results of another study that show *Sulfurimonas denitrificans* as the species most closely related to *A. butzleri* on the basis of a phylogenetic analysis of 60 genes taken from the available genomes of *A. butzleri* and members of *Campylobacteraceae* and other related bacteria (31).

The second, very recently published, genome is that of *A. nitrofigilis* (strain DSM 7299^T), the type species of the genus (124). The genome contains 3.19 Mb, and of the 3,224 genes predicted, 3,154 were protein-coding genes, of which 72.1% had a putative function (124). That publication provides an extensive description of the known phenotypic characteristics of these bacteria. It presents the general features of *A. nitrofigilis* according to the minimum information about a genome sequence and the number of genes associated with the general clusters of orthologous groups of proteins (124). However, this is the only information presented with no discussion on how the specific genes detected in the genome correlate with the specific physiological features. Furthermore, no reference is made to specific virulence genes or to a comparison with the well-defined features of the genome of *A. butzleri.* This missing information will probably be available from future studies. The genome of *A. nitrofigilis* (3.19 Mb) is larger than those of *A. butzleri* (2.34 Mb) and *C. jejuni* (1.64 Mb), which may indicate its adaptation to the environment rather than to a host (109). In contrast to the *A. butzleri* genome, which contains five rRNA operons with identical 16S rRNA sequences, four operons are present in *A. nitrofigilis* genome, showing differences of up to two nucleotides. This indicates that the 16S rRNA gene of *Arcobacter* also possesses microheterogeneities, as described for other genera (reference 4 and references therein).

At least two genome projects involving *Arcobacter* species are ongoing. At the 2009 International Workshop on *Campylobacter*, *Helicobacter*, and Related Organisms, results obtained from the draft genome of a bovine strain of *A. butzleri* that revealed a considerable divergence from the human strain RM4018 at loci involved in environmental sensing and survival were reported (138, 162). Furthermore, as indicated by Miller et al. (111), the genome of *A. halophilus* LA31B^T is also being sequenced, and the comparison of the draft genome of this bacteria with the data from *A. butzleri* (RM4018) have revealed that, despite expected common features, this species show multiple unique genes that requires further attention (109, 162). In fact, a preliminary analysis seems to explain the halophilic basis of the halotolerance of this species; in addition, the predicted proteins involved in arcobacters' aerotolerance have also been recognized (109).

CLINICAL IMPORTANCE

Arcobacter in Humans

The species *A. cryaerophilus*, originally identified in 1988 as *Campylobacter cryaerophila* (144), was the first isolated from a human specimen. Although the role of *Arcobacter* species in human diseases is not yet well established, *A. butzleri* and *A. cryaerophilus* have been associated with gastrointestinal diseases on several occasions both in population studies and in clinical cases, as shown in Tables 1 and 2 (1, 84, 95, 96, 132, 149, 153). Persistent watery diarrhea was the main symptom associated with *A. butzleri*, in contrast to the bloody diarrhea found in *Campylobacter jejuni* cases, with the rest of the mi-

TABLE 1. *Arcobacter* detection in and/or isolation from human fecal samples in population studies between 1991 and 2010

Country(ies)	Detection (technique) or isolation method (medium)[a]	Fecal samples[b]			*Arcobacter* species	Gastrointestinal symtomatology	Reference
		N	*n*	%			
USA and Europe	Direct detection (m-PCR)	201	16	8	*A. butzleri*	Diarrhea	84
Italy	Direct detection (m-PCR)	99	46	46.5	*A. butzleri*, *A. cryerophilus*	Asymptomatic[c]	46
	Enrichment (CAT broth)/isolation (CAT agar)	99	3	3	*A. butzleri*	Asymptomatic[d]	
France	Direct detection (real-time PCR)	345	4	1.2	*A. butzleri*	Diarrhea	1
	Enrichment (ASB)/isolation (ASM)	345	0				
Switzerland	Direct isolation (*Arcobacter* plating medium)	500	7	1.4	*A. cryaerophilus*	Asymptomatic[e]	76
South Africa	Direct detection (m-PCR)	322	35	11	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*	Diarrhea/asymptomatic	132
India	Direct isolation (*Campylobacter* blood agar)	400	5	1.25	*Arcobacter* spp.	Diarrhea	96
Belgium	Direct isolation (filtration and ASM)	67,599	77	0.1	*A. butzleri*, *A. cryaerophilus*	Diarrhea and asymptomatic	153
Hong Kong	Direct isolation (CMA)	4,741	6	0.13	*A. butzleri*	Diarrhea	99
South Africa	Direct isolation (filtration and CAT agar)	300	1	0.3	*A. butzleri*	Diarrhea	98
South Africa	Direct isolation (filtration on BA)	19,535	16	0.1	*A. butzleri*	Diarrhea	97
Denmark	Direct isolation (mCCDA)	1,376	2	0.1	*A. butzleri*, *A. cryaerophilus*	Diarrhea	40
England	Not specified	761	1	0.13	*A. cryaerophilus*	Diarrhea	146
Thailand	Direct isolation (filtration on BA)	631	15	2.4	*A. butzleri*	Diarrhea	142

[a] ASB, *Arcobacter* selective broth; ASM, *Arcobacter* selective medium; CMA, cefoperazone MacConkey agar; BA, blood agar; mCCDA, modified cefoperazone charcoal deoxycholate agar.
[b] *N*, number of samples studied; *n*, number of positive samples.
[c] Of the 46 positive samples, 30 (78.9% of 38) corresponded to type 2 diabetic subjects and 16 (26.2% of 61) corresponded to nondiabetic subjects.
[d] The three positive samples were from diabetics.
[e] All persons worked in slaughterhouses.

crobiological or clinical characteristics being very similar (153). In an *A. butzleri* outbreak affecting 10 children in an Italian school, the main symptom was recurrent abdominal cramps without diarrhea, and the infection was severe enough to require the hospitalization of 3 children (151). A study that investigated by molecular methods the prevalence of *Campylobacter*, *Helicobacter*, and *Arcobacter* in 322 stool specimens from patients (with and without HIV) in South Africa found *A. butzleri* to be the third most prevalent species (6.2%), after *Helicobacter pylori* (50.6%) and *C. jejuni* (10.2%) (132). In fact, in two independent studies performed in Belgium and France (127, 153), *A. butzleri* was the fourth most common *Campylobacter*-like organism recovered from stools of patients with diarrhea. Very recently, this species has also been found to be the etiological agent of traveler's diarrhea acquired by U.S. and European travelers to Mexico, Guatemala, and India, with a prevalence of 8% (84). That is the first study to demonstrate the association of *Arcobacter* with this type of infection.

Using culture methods, prevalence values reported for *Arcobacter* in diarrheic stool samples in population studies (Table 1) range from 0.1% in South Africa (97) to 2.4% in Thailand (142). However, using molecular detection methods, the prevalence was higher, ranging from 1.2% in France (1) to 12.9% in South Africa (132). The few studies carried out make it impossible to establish whether prevalence varies between developed and developing countries, although in the above-mentioned study of traveler's diarrhea, the prevalence ranged from 4% of visitors to Antigua (Guatemala) to 15% of those visiting Goa (India) (84). In all of the studies, *A. butzleri* tends to have the highest prevalence, followed by *A. cryaerophilus* and *A. skirrowii*, and the values reported by direct molecular detection by Samie et al. (132) were 6.2%, 2.9%, and 1.9%, respectively. So far, *A. skirrowii* has been associated with gastroenteritis on only a few occasions (132, 168). Among those affected were an elderly patient in whom it caused persistent diarrhea (168) and one schoolchild and five hospitalized patients (132).

The isolation of *Arcobacter* species from feces of healthy people has been reported in only a few studies (76, 132, 153). *A. cryaerophilus* was found in 1.4% of stool samples from asymptomatic workers in a slaughterhouse environment in Switzerland (76). Another study, conducted in Belgium, described human asymptomatic carriage of *A. butzleri* as being more frequent than that of *C. jejuni* but without significant differences (153). In South Africa, *Arcobacter* has also been detected in samples from asymptomatic people (8.8%; 14/160) but with a lower prevalence than in samples from people suffering diarrhea (12.9%; 21/162) (132). In the latter study, *Arcobacter* species were recovered from 55.1% (27/49) of the patients showing coinfection by two, three, or four pathogens, such as *C. jejuni*, *Campylobacter coli*, *Campylobacter concisus*, and *Helicobacter pylori*. The last species was found together with *A. butzleri* and *A. skirrowii* coinfecting two patients (132). This is relevant considering that the effects and dynamics of coinfections with multiple strains or species are still poorly understood.

Cases of bacteremia attributed to *A. butzleri* and *A. cryaerophilus* have also occasionally been reported, as shown in Table 2 (78, 100, 123, 167, 169). In one of these cases, *A. butzleri* was isolated from the blood of a neonate and the clinical history indicated an *in utero* sepsis, which was probably initiated by prenatal bleeding experienced by the mother, although the ultimate source of infection could not be established (123). This is the first indirect evidence of a possible vertical or transplacental transmission.

Although it has not been clearly established that host characteristics, such as age and immune status, play a role in *Arcobacter* infections, it is probable that they do, as this occurs with other microbes. Vandenberg et al. (153) described cases of diarrhea in infants and adults with ages ranging from 30 days to 90 years in Belgium, while Samie et al. (132) reported a lower prevalence *of Arcobacter* (3%; 2/67) in healthy schoolchildren aged 3 to 15 than in hospitalized children (10.4%; 12/115) with similar ages (ranging from 3 to 19 years old). In addition, in India, *Arcobacter* has been isolated in 1.5% of the feces samples from patients with diarrhea affected by HIV and in 1% of those from persons not affected by this disease (96). In contrast, Samie et al. (132) reported a higher

TABLE 2. Reports of human cases of *Arcobacter* spp., 1988 to 2004[a]

Country	Age of patient (sex)	Underlying disease(s) or condition(s)	Clinical symptom(s)	Species	Detection or isolation	Identification method	Reference
Chile	2 yr 6 mo (male), 1 yr (female)	Not specified	Chronic diarrhea	*A. butzleri*	Filter method	Biochemical	48
Belgium	73 yr (male)	Prosthetic aortic heart valve	Chronic diarrhea	*A. skirrowii*	Filter method	m-PCR	168
Hong Kong	69 yr (female)	Gangrenous appendicitis	Bacteremia	*A. butzleri*	Not specified	16S rRNA gene sequencing	100
Hong Kong	7 yr (male)	Suffocation in a mud pool	Bacteremia	*A. cryaerophilus*	Blood culture	16S rRNA gene sequencing	167
Taiwan	60 yr (male)	Chronic hepatitis B carrier, liver cirrhosis	Bacteremia	*A. butzleri*	Bactec 9240 aerobic bottle	16S rRNA gene sequencing	169
Taiwan	72 yr (female)	Chronic renal failure, hemodialysis with arteriovenous fistula	Bacteremia	*A. cryaerophilus*	Bactec 6A aerobic bottle	Biochemical. fatty acid analysis	78
	Neonate	Mother had prenatal bleeding	Bacteremia	*A. butzleri*		Biochemical	123
Germany	48 yr (male), 52y (female)	Diabetes mellitus I hyperuricemia, alcohol abuse	Diarrhea, abdominal cramps	*A. butzleri*	*Campylobacter* selective medium	Biochemical	102
Italy	3 to 7 yr[b]	Not specified	Abdominal pain, vomiting, fever	*A. butzleri*	*Campylobacter* selective medium	Polyphasic identification[c]	151
Unknown	2 yr (female)	Not specified	Gastroenteritis	*A. butzleri*	*Yersinia* selective agar	Not specified	16
Australia	35 yr (male)	Not specified	Chronic diarrhea	*A. cryaerophilus*	Blood agar with antibiotics	Polyphasic identification[c]	144

[a] No more cases have been reported since then.
[b] Ten children (6 girls and 4 boys).
[c] Biochemical, fatty acid, DNA-DNA hybridization, etc.

prevalence of arcobacters in HIV-negative (13.7%) than in HIV-positive (9.1%) patients. *Arcobacter* was also recovered from patients who showed other underlying diseases, such as type 1 and 2 diabetes mellitus (46, 102), liver cirrhosis (169), gangrenous appendicitis (100), or cancer and chronic renal failure (78), or people with an internal prosthesis (168) or hyperuricemia and alcohol abuse (102). Fera et al. (46) detected a high prevalence of *Arcobacter* fecal carriage in older subjects with type 2 diabetes but without gastrointestinal symptoms by multiplex PCR (m-PCR) (79%; 30/38); however, positive results by culture were much lower (7.9%; 3/38). The discordance found between these two methods is higher than that observed in other studies (26, 77).

Of all the above-mentioned studies, the ones that provide probably the strongest support for the potential role that *Arcobacter* species may have in the development of diarrhea are those of Vandenberg et al. (153) and Samie et al. (132). The former is the largest *Arcobacter* survey (studying 67,599 stool samples collected over 8 years); it used at least two isolation procedures and provides detailed clinical data from the patients (153). The latter describes a higher prevalence and diversity of arcobacters because it employed direct molecular detection from stool samples (132). However, it should be considered that so far no experimental infections with human volunteers have been attempted, nor has the development of an immunological reaction been tested, and these types of studies should be encouraged.

Although *Arcobacter* spp. are not currently considered microorganisms of major public health concern (139), data increasingly suggest that their significance in human infections may be underestimated, mainly because of inappropriate detection and identification methods (51, 139, 153). One of the major pitfalls is that the optimum growth conditions for recovery of *Arcobacter* (30°C) are generally not applied with clinical specimens. In fact, despite the fact that only some *A. butzleri* and *A. skirrowii* strains are able to grow at 42°C (121), this is the only temperature used for isolation of campylobacters in the majority of laboratories (1). Furthermore, campylobacters different from *C. jejuni* or *C. coli* and related organisms are rarely identified to the species level (153). Therefore, it is advisable in clinical research to use an additional culture medium incubated at 37°C to recover *Arcobacter* spp. in order to determine the true role and prevalence of these microbes in human disease. A more suitable approach may be to use an enrichment step (cefoperazone-amphotericin B-teicoplanin [CAT] broth or another medium) followed by passive filtration (0.45-μm filters) on blood agar (both incubated at 37°C for 48 to 72 h), combined, if possible, with molecular identification of as many colonies as possible in parallel to direct detection by PCR. This approach may be beneficial for providing information not only on the prevalence of arcobacters but also on the poorly known emergent campylobacters (non-*C. jejuni* and non-*C. coli*). This could be an intermediate solution while awaiting the availability of more information or more efficient and standardized isolation and identification protocols.

Arcobacter in Animals

Arcobacter has frequently been isolated from the intestinal tracts and fecal samples from different farm animals, but it apparently has the capacity to cause disease in only some of them (64). The most serious effects of *Arcobacter* in animals include abortions, mastitis, and diarrhea (104, 152). Although *Arcobacter* has been associated several times with bovine abortion (39, 115), these bacteria have also been recovered from healthy bovine preputial sheath washings (55), as well as from vaginal swabs from cows with no reproduction problems (89). Association with porcine abortion, with sows with reproductive problems, and with preputial fluid of boars and fattening pigs has also been reported (33, 115). *Arcobacter cryaerophilus* is the species predominantly linked to animal abortion, while *A. butzleri* and *A. skirrowii* are less frequent (33, 118). The recently described species *A. thereius* (74) was also recovered from livers and kidneys of spontaneous porcine abortions, but despite the fact that no other established abortifacient agents were detected, the pathogenic role of this recently described species still needs to be established beyond doubt (74).

Logan et al. (104) reported the isolation of an *Arcobacter* sp. isolate (then identified as an aerotolerant *Campylobacter*) from a milk sample during the course of an outbreak of mastitis in a dairy herd. In that study, four cows were experimentally infected by intramammary inoculation with the outbreak strain, and all of them developed an acute clinical mastitis that resolved spontaneously after 5 days. Another isolate recovered from the milk of a cow with mastitis was included among the strains used to describe the species *A. cryaerophilus* (115, 152). *Arcobacter butzleri* has been associated with enteritis and diarrhea in pigs, cattle, and horses, while *A. skirrowii* has been associated with diarrhea and hemorrhagic colitis in sheep and cattle (64, 152). Fecal shedding of *Arcobacter* is well known in poultry, i.e., chicken, ducks, turkeys, and domestic geese (9–11), although there have been no reports of any association with disease in those animals, and on that basis it has been suggested that poultry could be a natural reservoir of *Arcobacter* species (10, 65, 103). Some authors also consider pigs to be important hosts and reservoirs of *Arcobacter* species (68, 69).

In several studies *A. butzleri* has so far been the only species isolated both from healthy nonhuman primates (131, 141, 164) and from those with diarrhea (7, 63, 92). In one of these studies, the histological examination of the enteric tissues of the infected animals revealed a chronic active colitis (7). In other cases, the strains recovered showed a strong resistance to antibiotics (63, 141). Despite these data, the significance of arcobacters as a pathogen in nonhuman primates has yet to be determined.

Most clinical cases affecting animals are restricted to mammals, although one study (170) reported the isolation of *A. cryaerophilus* from a naturally infected rainbow trout (*Oncorhynchus mykiss*). The pathogenicity of the recovered strain was demonstrated by *in vivo* experimental infection, causing the death of the fish, which showed liver, kidney, and intestine damage.

Virulence Factors

The pathogenicity and virulence mechanisms of *Arcobacter* species are still poorly understood, despite several studies having investigated their adhesion capacity (17, 60, 66, 76, 114, 153), invasiveness (47, 66, 114, 153), and cytotoxicity (17, 60, 87, 114, 153, 158) in several cell lines (Table 3). Collectively, in those studies 56% (55/59), 20% (9/44), and 85% (164/194) of the strains tested showed adhesion, invasion, and cytotoxicity, respectively (Table 3), with toxicity and adherence therefore being the most commonly observed effects (17, 60, 66, 76, 87, 114, 153, 158). The differences observed among the different studies may be due to the origin of the strains (environmental versus clinical) as well as to different cell lines used in those studies (162). The capacity for *in vitro* invasion of cell lines has been demonstrated mainly for *A. cryaerophilus* (47, 66), while Wesley et al. (161) indicated that *A. butzleri* could be the most invasive species in experimental animal infections. The virulence of *A. cryaerophilus* was first described when it was observed that the strains tested induced the accumulation of fluid and electrolytes in the rat ileal loop assay and showed *in vitro* invasion of Hep-2 cells (47). This species seems to be more virulent for animals than the other species, because it is able to invade both the porcine intestinal tissues and the placenta, disseminating to the fetus, as demonstrated in a case of an infection in sows transmitted to their offspring (68). Other studies have demonstrated that *A. butzleri* strains colonized the intestines of all the experimentally infected piglets, but variable results were obtained for chickens and turkeys (161). It was also discovered that the Beltsville White turkey was the most suitable animal model for reproducing the diarrhea infection (160). Several other animal models and *in vivo* experiments have been reviewed in another recent publication (162). The presence of adhesion molecules in *A. butzleri* have been proven by the capacity of the strains tested to agglutinate human, rabbit, and sheep erythrocytes, and a hemagglutinin of about 20 kDa has been characterized by Western immunoblotting (147). This hemagglutinin is a lectin-like molecule, which is able to interact with erythrocyte receptors containing D-galactose (147). The mechanism by which *A. butzleri* induces diarrhea has been studied by infecting human colonic epithelial cells (HT-29/B6) (15). The results indicated that the process was mediated by a reduced expression of claudin-1, -5, and -8 tight-junction proteins, which generated an epithelial barrier dysfunction and apoptosis of those cells, resulting in a leak flux type of diarrhea (15). The induced expression of the proinflammatory cytokine interleukin-8 (IL-8) is considered a major virulence factor of *H. pylori* and *Campylobacter* spp. and has also been reported for *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. cibarius* (66). However, despite the fact that all tested *Arcobacter* strains show the ability to induce IL-8 production by human Caco-2 cells and porcine intestinal epithelial cells, no correlation with levels of cell adhesion or invasion was found (66). The disruption of tight junctions and the induction of a proinflammatory cytokine response in colonic epithelial cells have been also described as virulence factors in *C. jejuni* (21). The currently known virulence mechanism of *Arcobacter*, i.e., adhesion, toxin production, induction of inflammation, and an increase of the paracellular transport that lead to a watery diarrhea, is schematized in Fig. 2.

The above-mentioned evidence indicates that some *Arcobacter* species could indeed be enteropathogens. For instance, a watery diarrhea similar to that seen in humans was observed in naturally infected macaques (63, 162) and histological lesions compatible with colitis were observed in *A. butzleri*-infected animals, while no isolates from the feces of healthy animals were obtained (6). These results, together with data obtained from experimental animal models such as the Beltsville White turkey and the pig, where the same challenge mi-

TABLE 3. Summary of the pathogenicities of *Arcobacter* on different cell lines[a]

Species and strain origin	Cell line[b]	No. of positive samples/no. of tested samples			Reference
		Adhesion	Invasion	Cytotoxicity	
A. butzleri					
Seawater	Hep-2	6/17	ND[c]	ND	17
Human feces		12/12	4/12	3/12	153
Zooplankton		4/4	ND	ND	60
River water	HeLa	6/17	ND	ND	17
River water		1/8	0/8	ND	114
River water		ND	ND	3/3	87
Animal/human		ND	ND	3/3	87
Zooplankton		4/4	ND	ND	60
River water	INT407	1/8	0/8	ND	114
River water		ND	ND	3/3	87
Animal/human		ND	ND	3/3	87
Vero river water		ND	ND	17/18	114
Seawater		ND	ND	5/17	17
Meats		ND	ND	76/80	158
Zooplankton		ND	ND	3/4	60
River water	CHO	ND	ND	17/18	114
Human blood	Caco-2	1/1	0/1	ND	66
Human blood	IPI-2I	1/1	0/1	ND	66
Subtotal		36/72 (50%)	4/30 (13%)	133/161 (83%)	
A. cryaerophilus					
Swine feces	Hep-2	ND	1/1	ND	47
Bovine fetus		ND	1/1	ND	47
Human feces		4/7	ND	ND	76
River water	HeLa	ND	ND	3/3	87
Animal/human		ND	ND	3/3	87
River water	INT407	ND	ND	3/3	87
Animal/human		ND	ND	3/3	87
Vero meats		ND	ND	2/2	158
Porcine/ovine	Caco-2	4/4	2/4	ND	66
Human feces	Caco-2	2/7	ND	ND	76
Porcine/ovine	IPI-2I	4/4	1/4	ND	66
Subtotal		14/22 (64%)	5/10 (50%)	14/14 (100%)	
A. skirrowii					
Meats	Vero	ND	ND	17/19	158
Porcine/ovine	Caco-2	2/2	0/2	ND	66
Porcine/ovine	IPI-2I	2/2	0/2	ND	66
Subtotal		4/4 (100%)	0/2 (0%)	17/19 (89%)	
A. cibarius					
Chicken carcass	Caco-2	1/1	0/2	ND	66
Chicken carcass	IPI-2I	1/1	0/2	ND	66
Subtotal		1/1 (100%)	0/2 (0%)		
Total		55/99 (56%)	9/44 (20%)	164/194 (85%)	

[a] Data are from reference 64 and from this review.
[b] Hep-2, human larynx carcinoma cell line; HeLa, human cervical carcinoma cell line; INT407, human embryo intestinal epithelial cell line; CHO, Chinese hamster ovary cell line; Caco-2, human enterocyte-like cell line; IPI-2I, porcine epithelioid cell line.
[c] ND, not determined.

crobes that were able to cause diseases were recovered (160, 161), indicate that Koch's classical postulates have been partially fulfilled. Various aspects of arcobacter-associated gastroenteritis remain to be studied. For instance, it is so far unknown if animals and humans infected with *Arcobacter* strains have specific immunological responses, and this is essential evidence for establishing the true role of these organisms as gastrointestinal pathogens.

In comparison with the case for *Campylobacter*, almost nothing is known about which *Arcobacter* genes are involved in the virulence mechanisms. The recently published genome sequence of *A. butzleri* RM4018 revealed that this strain possesses some putative virulence determinants homologous to those of *C. jejuni*, such as the genes coding for fibronectin binding proteins CadF and Cj1349, invasin protein CiaB, putative virulence determinant MviN, phospholipase PldA, and hemolysin TlyA (110) and the major outer membrane protein PorA (109). All these genes except that for PorA were recently targeted by PCR and were found to be present in a set of 108 clinical and nonclinical *A. butzleri* strains (36). Furthermore, in that study, other genes found in the *A. butzleri* genome, i.e., those that encode HecB (a related hemolysin activator protein), HecA (a member of the filamentous hemagglutinin family), and IrgA (an iron-regulated outer membrane protein),

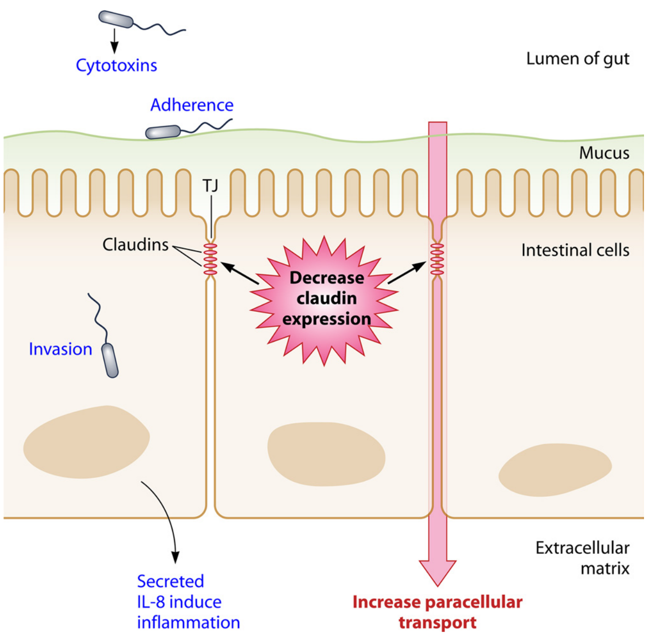

FIG. 2. Virulence mechanisms described for *Arcobacter* in different cell lines and exemplified for intestinal epithelial cells. Strains of *Arcobacters* species have shown the capacity to produce cytotoxicity, adherence, invasion, and inflammation mediated by interleukin-8 (IL-8). The ability to decrease claudin expression in tight junctions (TJ) with dysfunction of the epithelial barrier and the concomitant increase in paracellular transport, which leads to leak flux type of diarrhea, have been demonstrated for *A. butzleri* in human colonic epithelial cells (HT-29/B6).

were present in 60.99%, 25.82%, and 29.67% of the tested strains, respectively (36). However, it is still unknown whether these putative virulence determinants are functional or have roles similar to those of the *Campylobacter* homologs (110). The available genome sequence of *A. butzleri* does not contain the genes that encode the cytolethal-distending toxin (CDT), which is found in the genomes of various *Campylobacter* spp. (110) and produces cytotoxicity on eukaryotic cells by breaking the double stranded DNA. This corroborates previous findings by Johnson and Murano (87), who did not detect CDT genes by PCR in six *A. cryaerophilus* and 18 *A. butzleri* strains from different origins. However, Villarruel-López et al. (158) observed cytotoxic effects in Vero cells, such as the induction of vacuole formation and cell elongation, that were attributed to a toxin different from CDT. Similar cytotoxic effects were produced by *A. butzleri* strains in both Vero and CHO cells (114) and in several other cell lines (Hep-2, HeLa and INT407) (Table 3). However, the toxins involved have not yet been fully characterized, nor have their specific regulatory mechanisms or specific targets within the cell.

Lipopolysaccharides (LPSs), and particularly lipooligosaccharide (LOS), which are components of the outer membranes of bacteria, are known to play a major role in the host-bacterium interaction in *Campylobacter* (109). As indicated by Miller and Parker (109), *Campylobacter* cells alter their LOS and capsular polysaccharides via modulation of hypervariable polynucleotide tracts contained within contingency genes. However, such hypervariable tracts were not present in the *A. butzleri* strain RM4018 genome. Apart from this finding, the role of LPSs and LOS in *Arcobacter* has not been explored, and the only chemically characterized LOS corresponds to the halophilic marine bacterium *A. halophilus* (137).

Bacterial flagella and their protein subunits (flagellins) are involved in cell motility and chemotaxis and have a role in colonization and invasion of host cells, with flagellins being a primary target for the immune system (67, 109). Ho et al. (67) determined the sequences of the two flagellin genes (*flaA* and *flaB*) in strains of five species of *Arcobacter* and demonstrated by constructing mutants with mutations in either *fla* gene for one strain of *A. butzleri* that only *flaA* is necessary for motility. Flagellin genes of *Arcobacter* are not phylogenetically related to those of *Campylobacter* (67, 109, 162). Although the genome of *A. butzleri* RM4018 encodes all the flagellar structural genes, none of the flagellar transcription regulator genes such as sigma factors σ^{54} (RpoN), σ^{28}, and FlgM, which are found in other epsilonproteobacteria, are present (110). Instead, *Arcobacter* possess a large number of signal transduction systems, indicating that this organism is able to respond to many different environmental signals (110, 162). Miller and Parker (109) indicated that the functions of these missing genes could be assumed by those for other extracytoplasmic sigma factors found in the genome of *A. butzleri* but not in *Campylobacter*. Extracytoplasmic sigma factors can play a role in motility, as has been observed in *Myxococcus xanthus* (159). Despite this important new finding, it has not yet been elucidated whether in *Arcobacter* flagella can be considered an essential virulence factor for the colonization of the gastrointestinal tract, as occurs with *C. jejuni* (59).

New insights into the virulence of these microorganisms will soon be available by using the data from the available genomes of *A. butzleri* and *A. nitrofigilis* and from the other genomes that will soon be published (109). The recently developed tools for the construction of *Arcobacter* mutants (67) would probably be extremely useful for testing the role of potential virulence genes. As Wesley and Miller (162) indicated, a major challenge is identifying the virulence factors in this bacterial group, since these data are critical for establishing *Arcobacter* as a true pathogen, but of course this will have to be complemented with studies on the immunological response by the host.

Antibiotic Resistance

Like with *Campylobacter*, the majority of cases of enteritis and bacteremia caused by *Arcobacter* appear to be self-limiting and do not require antimicrobial treatment (69). However, the severity or prolongation of symptoms may justify the use of antibiotic treatment. In contrast to *Campylobacter* antimicrobial susceptibility tests, those for *Arcobacter* species are not standardized (169); a few studies using different methods, i.e., Etest, agar dilution, disc agar diffusion, or broth microdilution methods, have been carried out (43, 73, 90, 123, 140, 154). The results have shown that many *A. butzleri* strains are resistant to clindamycin, azithromycin, ciprofloxacin, metronidazole, carbenicillin, and cefoperazone (73, 123, 140, 143). Fluoroquinolones and tetracycline have been suggested for the treatment of human and animal infections produced by *Arcobacter* (140, 154) because they showed good activity against strains of several origins (43, 154). However, strains resistant to nalidixic acid and ciprofloxacin have been detected (2, 123, 143). So far it had been demonstrated that two *A. butzleri* strains and one *A. cryaerophilus* strain that were resistant to ciprofloxacin (with MICs ranging from 6 to 12 mg ml^{-1}) show a mutation in the quinolone resistance-determining region of the *gyrA* gene (2), which could also be present in other strains. On the other hand, the *A. butzleri* strain (RM4018) from which the complete genome was sequenced showed a high antibiotic resistance associated with the presence or absence of specific genes that regulate antibiotic susceptibility (110). Regarding this, the presence of the *cat* gene (encoding a chloramphenicol *O*-acetyltransferase) was related to chloramphenicol resistance, three putative β-lactamase genes or the *lrgAB* operon was associated with β-lactam resistance, and the absence of the *upp* gene (encoding uracil phosphoribosyltransferase) was associated with 5-fluorouracil resistance (110).

It is important to note that there have only been a few antimicrobial susceptibility studies with clinical *Arcobacter* strains (recovered from human or animal cases), and in the absence of a specific recommended treatment, treatment is empirical. This was emphasized in the case of the neonate bacteremia, mentioned above, where the effectiveness of the therapy given was considered fortuitous in view of the multiple antibiotic resistance displayed by the isolated strain of *A. butzleri* (123). It has been suggested that infections produced by *Arcobacter* spp. probably require a treatment different from that applied to infections produced by the common *Campylobacter* species (153). Therefore, there is a need to perform these tests in order to establish the most adequate treatment in each specific case.

TRANSMISSION ROUTES

Consumption of *Arcobacter*-contaminated food or water is considered the route of transmission to human and animals, although this has not yet been proven (64, 111). In some drinking water outbreaks *Arcobacter* spp. have been isolated from the patients and/or from the contaminated water (53, 95, 129). In addition, *Arcobacter* species have been defined as potential zoonotic agents due to their pathogenic role in humans and animals (19, 64), and using an evidence-based semiquantitative method for prioritization of food-borne zoonoses, *A. butzleri* was ranked as a microbe of significant importance (19). Direct transmission between these two groups has not yet been demonstrated. In a detailed review by Cervenka (20), the physical and chemical treatments that can be applied for the control and elimination of arcobacters from food and water were evaluated. On the basis of the reviewed studies, it was concluded that *Arcobacter* strains tolerate high sodium chloride concentrations, grow at lower refrigeration temperatures, have the ability to attach to various types of surfaces, and are not very susceptible to desiccation. All these characteristics may explain the fate of these microbes in food products. However, it has been demonstrated that a heat (50°C) followed by cold shock (4 or 8°C) produces a lethal synergistic effect, reducing more *Arcobacter* cells than an individual treatment at 50°C or a cold shock temperature of 12°C or 16°C (38).

Up to now, no clinical isolate has been matched (genetically) with environmental isolates.

Person-to-Person Transmission

Person-to-person (PTP) transmission of *A. butzleri* was suggested to have occurred in an outbreak of recurrent abdominal cramps in an Italian school (151). The epidemiological data showed that all strains recovered from fecal samples from the infected patients (10 children) had the same phenotype and genotype (150). Another possible PTP transmission, mentioned above, was associated with a neonate, who was presumably infected through the placenta with *A. butzleri* (123).

Arcobacter in Water

As noted above, water is one possible route of transmission of arcobacters to animals and humans (reference 64 and references therein). In fact, strains of *Arcobacter* isolated from drinking water treatment plants in Germany had the same serotypes as those observed for human isolates (82, 83). However, while this could be suggestive of transmission, it was not definitively proven by genotyping the strains. Furthermore, members of this genus have been recovered from several types of environmental waters, i.e., rivers, lakes, groundwater, and seawater, as well as from plankton (26, 44, 113, 129). It was recently hypothesized that *Arcobacter* species are autochthonous to aquatic environments (44), although a high prevalence of these bacteria has also been observed in feces of livestock animals (157) and in farm effluents (22). This could indicate that those are the sources of surface water contamination. In fact, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* are significantly more prevalent in water that is fecally contaminated than in water that is not (26). These species enter seawater with the polluted freshwater, where they are probably able to coexist with other indigenous species, such as *A. marinus* or *A. halophilus*, which have so far been isolated only in those environments (26). Furthermore, all sewage samples studied were positive for *Arcobacter* and showed a great diversity of species (26), even a new candidate species such as *A. defluvii* (28), indicating that sewage may be an important reservoir for these microbes. The population structure of microorganisms in the sewage water inflow to wastewater treatment plants in Milwaukee (WI) was recently investigated using pyrosequencing of hypervariable regions in the 16S rRNA genes, and a great number of *Arcobacter* sequences were found, in contrast to the few detected in surface waters (108). That study indicated that further work is needed to determine if these microorganisms could be residents growing in the sewer systems. This may explain why these microbes are so abundant in human sewage, to the extent that they can be isolated and detected without the need for an enrichment step (26), which is in contrast to the low prevalence found in human feces. Its high abundance in sewage could also be explained by the adaptation of *Arcobacter* to the cooler temperature of the sewage systems, a more "natural" habitat than the human host, but this remains to be demonstrated.

As mentioned above, the information derived from the genome indicates that arcobacters are free-living, waterborne microbes able to adapt to very diverse environments (162), and as such it is not strange that they have been associated with at least three drinking water outbreaks (53, 95, 129). The first was an occurrence of gastroenteritis at a Girl Scout camp in Idaho (with nausea, vomiting, abdominal cramps, and diarrhea as the predominant symptoms) that affected 81% of those present (79% staff and 80% campers) (129). *Arcobacter butzleri* (misidentified at first as *Campylobacter jejuni* as indicated in the Panhandle Health District report) was the only microbe isolated from the well water used as the source of drinking water, and it was assumed to be the source of the outbreak because at that time the automated chlorination system for the camp drinking water had broken down (129). This was in fact the first U.S. report of *Arcobacter butzleri* from groundwater (129). The second outbreak, which caused 1,450 cases of cramps and diarrhea as the predominant symptoms, was reported in South Bass Island (Ohio) and had multiple etiologies (i.e., different microorganisms were isolated from the tested stools) (53), but despite *Arcobacter* not being recovered from the stools, it were isolated in the most fecally polluted well water samples. Finally, *A. cryaerophilus* and other different pathogens were isolated from stool samples of patients in Slovenia during an outbreak caused by contamination of the drinking water system after it had been connected to a new building (95). However, in none of those outbreaks it was fully proven that *Arcobacter* was the etiological agent. All these outbreaks were related to the presence of fecal contamination, but the capacity of *Arcobacter* to adhere to different types of pipes and to form biofilms (8) should be considered. Although the susceptibility of *A. butzleri* to chlorine has been demonstrated (20, 112, 129), it was not known if conventional procedures for drinking water treatment could effectively remove this bacterium, as described by Ho et al. (64). In a recent study it was found that although the species *A. butzleri* and *A. cryaerophilus* were very prevalent in the Llobregat River water (one of the main sources of drinking water production for the metropolitan area of Barcelona, Spain), these species were never detected or isolated from finished drinking water, clearly demonstrating that water treatment is effective in removing *Arcobacter* species (27).

Van Driessche and Houf (156) demonstrated that the capacity of *Arcobacter* species to survive in water is influenced by the presence of organic matter and temperature, showing that under laboratory conditions *Arcobacter* can stay viable for up to at least 250 days at 4°C. The loss of culturability of this species in nonchlorinated water stored at 12°C occurs after 21 days (112), while according to other authors, it occurs after 3 to 4 weeks on agar plates at 4°C (20). Moreover, it has been reported that *A. butzleri* has the ability to become viable but nonculturable (VBNC) when subjected to different laboratory conditions (20, 45), but this VBNC state has not yet been shown to occur in *Arcobacter* in natural aquatic environments.

Arcobacter in Foods

As mentioned above, food products of animal origin have also been suggested as an important potential transmission route of *Arcobacter* (23, 54, 64, 81, 101, 135, 139). This hypothesis relies on the high prevalence of those microbes in the intestinal tract and fecal samples of healthy farm animals and in many retailed meat products (6, 22, 64, 89, 101, 157). The results on the prevalence of *Arcobacter* found in 15 studies that investigated chicken, pork, and beef meat have been summarized previously (25). However, this summary did not include studies of carcasses, viscera, or skin, where these microbes are also abundant (6, 11, 49, 72, 134). It has been indicated that contamination of meat products by *Arcobacter* probably occurs when the feces of contaminated animals comes into contact with the carcasses during the slaughtering process (12). Most studies on the prevalence of *Arcobacter* in foods are on poultry (which has the highest prevalence), followed by pork, beef products (reference 25 and references therein), and raw milk (135). In the case of poultry, there has been some controversy about the origin of the contamination, because some authors suggested that the slaughter environment and not the feces were the source because *Arcobacter* could not be isolated from the feces (155). However, other authors later showed that these microbes inhabit the chicken intestine, indicating that the age of the sampled animals and the method used for recovery and identification influence the prevalence (65). Survival tests on some *Arcobacter* isolates in tap water at a scalding temperature of 52°C for 3 min, carried out by Ho et al. (65), indicated that a proportion of the arcobacters that contaminated the scalding water were able to survive these conditions and cause cross-contamination within and between flocks in the scalding tank and in later processing stages.

Shellfish are another potential source of infection according to the few existing studies (49, 106). In a study that investigated 84 samples of shellfish (shrimp, mussels, clams, and oysters), 100% of the clams and 41.1% of the mussel samples contained a high prevalence and a wide diversity of *Arcobacter* species (25). Mussels were the origin of the new species *A. mytili* and *A. molluscorum* (24, 52). This could have some public health importance considering that seafood is traditionally often eaten undercooked or raw. Very recently, it was shown that *Arcobacter* is found not only in raw food products but also in meals at popular restaurants in Bangkok, with a higher prevalence than other common enteropathogens, such as *Salmonella* and *Campylobacter* (143). It was established that the risk of exposure per consumed meal was 13%, and it was up to 75% in the case of 10 meals or more (143).

Studies on foods have shown that, in general, *A. butzleri* is the most prevalent species, followed by *A. cryaerophilus* and *A. skirrowii*, as reviewed by Lehner et al. (101) and further demonstrated in other recent studies (25, 125, 135). This has probably been the reason for the inclusion of *Arcobacter butzleri* in the list of microbes considered a serious hazard to human health by the International Commission on Microbiological Specifications for Foods (81). However, it should be taken into consideration that more than one *Arcobacter* sp. can normally be isolated in food products (25, 71, 125, 155). Less commonly isolated species are *A. nitrofigilis* (25, 107) and *A. mytili* and *A. thereius* (25). As indicated above, Cervenka (20) and D'Sa and Harrison (38) have reviewed several treatments and conditions that may help to eliminate or control the presence of arcobacters from food products, as Houf (69) recently did for the methodologies of isolation, identification, and genotyping.

Arcobacter in Pets and Wild Animals

Contact with pets' feces or by licking are other potential routes of transmission of *Arcobacter*. Very recently, Fera et al. (42), using the m-PCR developed by Houf et al. (77), reported high prevalences of *A. butzleri* (77.6%) and *A. cryaerophilus* (34.1%) in oral swab samples from pet cats. They suggested that the presence of arcobacters in these pets may play a role

TABLE 4. Media and procedures used for isolation of *Arcobacter* from different types of samples[a]

Enrichment			Isolation			
Semisolid or broth medium	Selective antibiotic(s) (concn, mg/liter)	Incubation conditions	Plating medium	Selective antibiotic(s) (concn, mg/liter) or procedure	Incubation conditions	Reference(s)
EMJH	5-Fluorouracil (100)	30°C, 48-72 h, mO_2	Blood agar	No antibiotics	30°C, 48-72 h, mO_2 and O_2	39
ASB	Cefoperazone (32), piperacillin (75), trimethoprim (20), cycloheximide (100)	24°C, 48 h, O_2	ASM	Cefoperazone (32), piperacillin (75), trimethoprim (20), cycloheximide (100)	24°C, 48-72 h, O_2	30
EMJH	5-Fluorouracil (200)	30°C, 9 days, O_2	CVA	Cephalothin (20), vancomycin (10), amphotericin B (5)	30°C, 48-72 h, mO_2	29
CAT broth[b]	Cefoperazone (8), amphotericin B (10), teicoplanin (4 mg/)	30°C, 48 h, mO_2	Blood agar	No antibiotics, membrane filtration	30°C, up to 7 days, O_2	9
JMB	Cefoperazone (32), 5-fluorouracil (200)	30°C, 48 h, O_2	JM agar	Cefoperazone (32)	30°C, 48 h, O_2	85, 86
Arcobacter broth[c]	Cefoperazone (16), amphotericin B (10), 5-fluorouracil (100), novobiocin (32), trimethoprim (64)	28°C, 48 h, mO_2	*Arcobacter* plating medium	Cefoperazone (16), amphotericin B (10), 5-fluorouracil (100), novobiocin (32), trimethoprim (64)	30°C, 24-72 h, mO_2	72

[a] EMJH, Ellinghausen-McCullough-Johnson-Harris semisolid medium; ASB, *Arcobacter* selective broth; ASM, *Arcobacter* selective medium; CVA, cephalotin, vancomycin, and amphotericin B agar; JMB, Johnson-Murano broth; JM, Johnson-Murano; O_2, aerobic conditions; mO_2, microaerobic conditions.
[b] Also called *Arcobacter* enrichment broth supplemented with CAT (cefoperazone, amphotericin B, and teicoplanin).
[c] Van Driessche et al. (157) modified the selective supplement of *Arcobacter* broth by adding cycloheximide (100 mg/liter) and increasing the novobiocin concentration to 64 mg/liter.

in their dissemination in the domestic habitat. However, in a previous study conducted in Belgium, Houf et al. (70) reported no isolation from oral swabs or cat feces. Despite that, in the latter study *A. cryaerophilus* was isolated from feces (1.5%) and oral swabs (0.7%) of dogs, while *A. butzleri* was recovered only from fecal samples (0.75%). A prevalence of 3.3% in feces of dogs in Chile was reported by Fernández et al. (50). However, Aydin et al. (12) did not find any positive fecal samples from dogs in Turkey. These differences in prevalence could be due to the different methods of isolation and detection of *Arcobacter* spp. used in those studies.

Few studies have been carried out to determine the presence of *Arcobacter* species in wild animals, as indicated by Hamir et al. (61), who reported the presence of *Arcobacter* spp. in 6 of the 10 intestinal samples from raccoons (*Procyon lotor*) studied. They suggested that these animals might play a significant role in the epidemiology of these bacteria, since they share the urban or suburban environment with humans. However, no studies are yet available for birds, which play an epidemiological role with *Campylobacter*. Moreover, *Arcobacter* has also been reported to be present in other exotic or nondomesticated animals, such as the Galapagos turtle, the black and white rhinoceros, the gazelle, the rhea, and the alpaca (162, 164).

Transmission among Animals

Vertical or transplacental *Arcobacter* transmission has been demonstrated from carrying sows to their offspring, as has horizontal or postnatal infection of piglets from their mothers, newcomers, or the environment (68).

Recently, Ho et al. (65) found a high prevalence of *Arcobacter* in the intestinal contents of poultry. In that study, the isolates recovered from the contents of the gut and from the carcasses of the same flock had similar genotypes (determined using enterobacterial repetitive intergenic consensus-PCR [ERIC-PCR]). In addition, it has been demonstrated that the intestinal tracts and oviducts of breeding hens can be infected with *Arcobacter*, although no evidence for transmission from hens to eggs was found (103).

ISOLATION AND DETECTION

Despite the fact that various media and procedures have been used to isolate *Arcobacter* from different samples (Table 4), a standardized reference method has not been proposed so far. The first isolate of *Arcobacter* (at that time called a *Spirillum*/*Vibrio*-like organism) was recovered in 1977 from aborted bovine fetuses by using the Ellinghausen-McCullough-Johnson-Harris (EMJH) *Leptospira* medium (39). One of the most commonly employed *Arcobacter* isolation protocols is based on the use of an enrichment broth supplemented with cefoperazone, amphotericin B, and teicoplanin, known as CAT broth, followed by passive filtration of the broth through a 0.45-μm filter placed over blood agar (9). Johnson and Murano (85, 86) proposed a new enrichment broth and isolation medium with cefoperazone and 5-fluorouracil as selective supplements and achieved good recovery of *Arcobacter* and strong inhibition of other bacteria. Another very popular method was designed after an antimicrobial *Arcobacter* susceptibility study by Houf et al. (72) and consists of a selective isolation protocol that incorporates five antibiotics in both the enrichment and the plating medium. This is the only method that has been validated for fecal specimens, by evaluating the recovery of arcobacters from artificially contaminated fecal samples (76). Van Driessche et al. (157) later modified the enrichment and the plating medium described by Houf et al. (72) for the isolation of these bacteria from animal fecal specimens, adding cycloheximide (100 mg l^{-1}) and increasing the novobiocin concentration (from 32 to 64 mg $liter^{-1}$). Recently, Scullion et al. (135), employing the methods of Houf et al. (72) and Johnson and Murano (85) in parallel, obtained 25% more positive samples from packed retail poultry than when using each method independently.

The most common procedures for the isolation of *Arcobacter* and other campylobacters from human clinical samples are the combination of sample filtration over an antibiotic-free blood agar plate (to eliminate larger accompanying microbes) and inoculation of the samples directly onto a selective medium in parallel (1, 97, 98, 153, 154). However, the use of an enrichment step before filtration increased *Campylobacter* detection by 38.5% (165), and it is likely that the same could occur for arcobacters. However, it has been reported that the enrichment step reduces the diversity of *Arcobacter* species recovered in the plating medium in comparison with direct plating (68, 71). In summary, it can be said that the methods for recovering *Arcobacter* are very diverse (Table 4) and that there is a lack of consensus about which of them is the most useful (depending on the type of sample), because few comparative studies have been performed (6, 85, 134) and, to our knowledge, there is no study in which they were all compared simultaneously. Some of the recovery problems reported include the inhibition of some *Arcobacter* species when using certain antibiotics (11, 72) and insufficient inhibition of the accompanying microbiota (6, 44). Generally, although not in clinical microbiology, *Arcobacter* isolation includes an enrichment step (which usually takes 48 h) in a broth containing several antibiotics followed by isolation on agar media (with or without antibiotics) for an additional incubation period of 48 to 72 h (Table 4). As mentioned above, some studies indicate that the enrichment step reduces the diversity of species because it favors the faster-growing species (68, 71). This may also affect their direct molecular detection from enrichment broth. Regarding this, Ho et al. (68), using the primers of the m-PCR for the simultaneous detection and identification of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* developed by Houf et al. (77) but performing individual PCRs for each species, demonstrated that only the predominant species would be detected from the broth. This is because amplification is favored for templates with a higher concentration in the PCR mixtures (68). However, direct detection from the CAT broth by m-PCR and identification of isolates recovered by culture after passive filtration of the broth on blood agar (without any antibiotic supplement) produced more or less the same results in other studies (23, 25, 26). This concordance agrees with results obtained by Houf et al. (77). In contrast, other authors have found discordant results between molecular detection and culturing (41, 44, 46, 56). This could be due to the different protocols used for culturing, which included a shorter enrichment period (24 h instead of 48 h) and/or the use of a plating medium with insufficient inhibition over other bacteria because no filtration was used (26). Moreover, coexisting strains or species may have been missed when picking only a few colonies from the isolation plates (64). Another poorly explored aspect is the need (or not) for microaerophilic conditions for the recovery of *Arcobacter* from both clinical and environmental samples. Reviewing the available data in the literature, around half of the studies used aerobic conditions in the isolation procedures (Table 4). Only one study used both aerobic and microaerophilic conditions in parallel, but this study generated inconclusive results (56).

Several molecular detection methods, aimed at improving sensitivity and reducing the time required for conventional methods, have been developed for *Arcobacter*. The most globally used method is the above-mentioned m-PCR (targeting the 16S or 23S rRNA gene) developed in 2000 by Houf et al. (77). Additionally, at least three real-time PCRs have been designed, using TaqMan (14), fluorescence resonance energy transfer (1), and SYBR green (58) technologies. When the latter two methods were applied to food and water samples and results were compared with those of the currently used m-PCR (77), they provided a 2-log-unit improvement in sensitivity (14, 58). On the other hand, a DNA microarray targeting housekeeping and virulence-associated genes has been developed for the detection of *A. butzleri*, *C. jejuni*, and *C. coli* and has shown a high level of specificity and sensitivity (128). Some genus-specific PCR assays targeting the 16S rRNA gene (62) or the 23S rRNA gene (13) have been described, but false-negative reactions have been reported for the latter (135).

IDENTIFICATION

Phenotypic Identification

Arcobacter spp. can be differentiated from *Campylobacter* spp. by their ability to grow in air and at lower temperatures ranging from 15 to 30°C (116, 148, 152). However, they show morphological characteristics similar to those of *Campylobacter*, i.e., Gram-negative, slender, spirally curved rods, which are often S shaped or helical and motile by means of a single polar unsheathed flagellum at one or both ends of the cell (31, 148, 152). Using only phenotypic or biochemical methods, these two genera could be confused, as indicated by Yan et al. (169) and González et al. (57). However, this can be avoided by checking the aerotolerance and growth at 15, 25, and 37°C. In general, classical biochemical tests routinely used for the identification of clinical bacteria often yielded negative or variable results with *Arcobacter* species (31). On et al. (119–121) standardized the inoculum and biochemical identification tests for campylobacters, and from their results a set of tests useful for distinguishing *Arcobacter* species were proposed in *Bergey's Manual of Systematic Bacteriology* in 2005 (148). However, eight new *Arcobacter* species have been described since then (24, 28, 34, 35, 52, 74, 75, 94). Table 5 shows the most useful biochemical tests for differentiating the currently accepted and recently proposed *Arcobacter* species.

Molecular Identification

Due to the difficult phenotypic characterization of *Arcobacter* spp., several molecular methods have been designed for its identification at the species level (Table 6). In fact, several

TABLE 5. Phenotypic characteristics of all *Arcobacter* species[a]

Characteristic	*A. nitrofigilis*	*A. cryaerophilus*	*A. butzleri*	*A. skirrowii*	*A. cibarius*	*A. halophilus*	*A. mytili*	*A. thereius*	*A. marinus*	*A. trophiarum*	*A. defluvii*	*A. molluscorum*
Enzyme activity												
Catalase	+	+	V	+	V	−	+[b]	+	−	+	+[b]	+
Urease	+	−	−	−	−	−	−	−	−	−	+	−
Nitrate reduction	+	+[c]	+	+	−	+	+[d]	+	+	−	+	+[e]
Indoxyl acetate hydrolysis	+	+	+	+	+	+	−	+	+	+	+	−
Growth conditions												
O_2 at 37°C	V	V	+	+	−	+	+	−	+	−	+	+
mO_2 at 37°C	−	V	+	+	+	+	+	−	+	−	+	+
1% (wt/vol) glycine	−	−	−	−	−	+[f]	+	+	+[f]	V[g]	−	−
4% (wt/vol) NaCl	+	−	−	+	−	+	+	−	+	−	−	+
MacConkey agar	−	V	+	−	+	−[f]	+	V	−[f]	V[h]	+	+
Minimal medium	−	−[i]	+	−	+	−[f]	−	+	−[f]	−[g]	+	−
Resistance to cefoperazone (64 mg $liter^{-1}$)	−	+	+	+	+	−[f]	−	+	−[f]	+	V	+

[a] Data are from references 24, 28, 34, 35, 52, 74, 75, 94, and 121. +, ≥95% of strains positive; −, ≤11% of strains positive; V, 12 to 94% of strains positive. O_2, aerobic conditions; mO_2, microaerobic conditions.
[b] Weak reaction (24, 28).
[c] Two of the four strains tested by Collado et al. (24) (LMG 9904^T and LMG 9065) were negative.
[d] Nitrate reduction was found to be positive for the three strains of *A. mytili*, in contradiction to previously published data (24, 52).
[e] Nitrate is reduced after 72 h and 5 days for all strains under microaerobic and aerobic conditions, respectively (52).
[f] Data from reference 52, all tested in medium supplemented with 2% NaCl.
[g] Test not evaluated by De Smet et al. (34) and tested by Figueras et al. (52) ($n = 3$).
[h] Strains LMG 25534^T and LMG 25535 of *A. trophiarum* and strain FE2 (CECT 7650) of this species grew on MacConkey agar (52), in contrast to the 80% positive response described for this species (34).
[i] Two of the four strains tested by Collado et al. (24) (LMG 7537 and LMG 10241) were positive.

different and specific PCR protocols were necessary for the identification of *Campylobacter* and *Arcobacter* species recovered from human feces (132). Again, the most globally used method is the above-mentioned m-PCR developed by Houf et al. (77). Although this method is very popular, it produces misidentification of *A. nitrofigilis* with *A. skirrowii* and also confuses the latter species with the recently proposed species *A. mytili* (24) because of the identical amplicons obtained. Furthermore, the recently described species *A. thereius* is also confused with *A. cryaerophilus* (25, 37), as occurs for *A. defluvii* and *A. mollucorum* (28, 52). In 2003, Kabeya et al. (88) proposed another m-PCR for the identification of the species considered to be of medical importance, i.e., *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, which have been used in some studies for the characterization of isolates recovered from fecal and food samples (90, 113, 140). This method was also designed for differentiating the two groups (1A and 1B) of *A. cryaerophilus*, but recent studies indicated that this differentiation was not always possible (37) or meaningful (32). Several additional molecular methods for the detection and/or identification of *Arcobacter* spp. have recently been described (1, 4, 14, 126, 128). These methods use different technologies, i.e., PCR-denaturing gradient gel electrophoresis (PCR-DGGE) (126), real-time PCR (1, 14), the DNA microarray mentioned above (128), and matrix-associated laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry (3). None of them detected or identified all the accepted *Arcobacter* species, because not all species were tested (Table 6). In 2008, a 16S rRNA gene-RFLP method that uses a digestion with the MseI enzyme and produces species-specific patterns for all the species described at that time (*A. butzleri*, *A. cryaerophilus*, *A. cibarius*, *A. skirrowii*, *A. nitrofigilis*, and *A. halophilus*) was described (51). This method not only has been successfully used for the identification of more than 600 *Arcobacter* strains in several studies (23, 25–27, 51) but also has made it possible to recognize new *Arcobacter* species mostly by their new RFLP patterns, such as *A. mytili* (24), *A. defluvii* (28), and *A. molluscorum* (52), among others that are waiting to be described (25). This method can also differentiate the new species *A. marinus*, because a distinctive novel pattern has been observed both after *in silico* simulation and experimentally (52). The proposed 16S rRNA gene-RFLP method (51) cannot, however, differentiate the recently described species *A. thereius*, because it produces the same pattern as *A. butzleri* (25, 37). This shortcoming, together with the fact that the method uses polyacrylamide gel electrophoresis, was considered the main obstacle for its routine use (37). However, the species *A. thereius* and *A. butzleri* can be easily separated by phenotypic methods (Table 5) or by a very recently proposed m-PCR method (37). This new method uses seven primers and can differentiate all the accepted species to date except *A. nitrofigilis*, *A. mytili*, and *A. halophilus*. Its usefulness for *A. marinus* is unknown because the description of that species was in press at the time that the method was developed. However, the recently proposed species *A. trophiarum* cannot be differentiated with this new m-PCR method (37), but it can be recognized on the basis of a PCR assay targeting the *hsp60* gene (34). When this new m-PCR method (37) was tested for the new candidate species, *A. defluvii* produced the same expected band as *A. butzleri*, and no amplicon was generated for *A. molluscorum* (28, 52). Therefore, the response of these two

TABLE 6. Comparison of molecular methods for identifying *Arcobacter* spp.

Reference	Method[a]	Gene(s) targeted	Species discriminated	Comment
93	RFLP, Southern blotting	16S rRNA, 23S rRNA	*A. butzleri*	Equal patterns for *A. cryaerophilus* and *A. skirrowii*
18	PCR-RFLP	16S rRNA	*A. butzleri*	Equal patterns for *A. cryaerophilus*, *A. skirrowii*, and *A. nitrofigilis*
62	Multiplex PCR	16S rRNA, 23S rRNA	*Arcobacter* sp., *A. butzleri*	Equal patterns for *A. cryaerophilus* and *A. skirrowii*
80	PCR-RFLP	23S rRNA	*A. butzleri*, *A. nitrofigilis*	Equal patterns for *A. cryaerophilus* and *A. skirrowii*
105	PCR-RFLP	16S rRNA	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*	
77	Multiplex PCR	16S rRNA, 23S rRNA	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*	Confusion with some species has been reported by Figueras et al. (51, 52), Collado et al. (24, 28), and Houf et al. (74)
5	PCR-hybridization	*glyA*	*A. butzleri*	
88	Multiplex PCR	23S rRNA	*A. butzleri*, *A. cryaerophilus* 1A, *A. cryaerophilus* 1B, *A. skirrowii*	DNA concn must be accurately adjusted to 20 ng/reaction because any difference in the concn of the template produces some nonspecific amplifications (88)
91	PCR-RFLP	*groEL*	*A. butzleri*	No other *Arcobacter* species were tested
57	PCR-RFLP	16S rRNA, 23S rRNA	*A. butzleri*	Equal patterns for *A. cryaerophilus* and *A. skirrowii*
126	PCR-DGGE	16S rRNA	*A. cryaerophilus* 1B, *A. nitrofigilis*	Equal patterns for *A. cryaerophilus* 1A, *A. butzleri*, and *A. skirrowii*
14	Real time PCR	*rpoBC*, 23S rRNA	*A. butzleri*, *A. cryaerophilus*	Not tested in other studies
	Multiplex PCR	*rpoBC*, 23S rRNA	*A. butzleri*, *A. cryaerophilus*	The m-PCR uses primers CRY1-CRY2 described by Houf et al. (77), for which unspecific reaction has been reported (74)
1	Real time PCR	*gyrA*	*A. butzleri*, *A. cryaerophilus*, *A. cibarius*, *A. nitrofigilis*	Failed for the identification of *A. skirrowii*
51	PCR-RFLP	16S rRNA	*A. butzleri*, *A. cryaerophilus* 1A, *A. cryaerophilus* 1B, *A. skirrowii*, *A. cibarius*, *A. nitrofigilis*, *A. halophilus*, *A. cibarius*, *A. mytili*	The new species *A. thereius* (74) showed the same restriction pattern as *A. butzleri*; a specific pattern was obtained for *A. marinus* (52) and also for the new species *A. defluvii* and *A. molluscorum* (28, 52)
125	PCR	*gyrA*, 16S rRNA	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius*	Tested in a limited no. of strains and routine used should be further evaluated, according to Douidah et al. (37)
3	MALDI-TOF MS	Proteins	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*	Only 3 *A. butzleri* strains and a single strain each of *A. cryaerophilus* and *A. skirrowii* were tested
37	Multiplex PCR	23S rRNA, *gyrA*	*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius*, *A. thereius*	The new species *A. defluvii* produced the same band as *A. butzleri* (28)
34	PCR	*hsp60*	*A. trophiarum*	

[a] RFLP, restriction fragment length polymorphism; DGGE, denaturing gradient gel electrophoresis; MALDI-TOF MS, matrix-associated laser desorption ionization–time-of-flight mass spectrometry.

new species should be taken into consideration when applying this m-PCR method in future studies.

Considering all the above-mentioned limitations of individual methods, an approach that produced good results for the identification of *Arcobacter* is the application of the 16S rRNA gene-RFLP method (51) in parallel with the m-PCR method (77), because when incongruent results between the two methods were found, final identification was performed by sequencing the 16S rRNA gene (23, 24, 28, 51, 52).

GENOTYPING

Different methods have been used for distinguishing one strain of *Arcobacter* from another, for studying transmission routes, or for tracing sources of outbreaks, including several PCR methods, such as enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), randomly amplified polymorphic DNA-PCR (RAPD-PCR) (71), AFLP (117, 118), and pulsed-field gel electrophoresis (PFGE) (79). Each of these methods has advantages and disadvantages related to its reproducibility, simplicity, discriminatory power, and cost (71). The most commonly used typing technique has been ERIC-PCR, which has been successfully applied for the investigation of outbreaks (150), for the characterization of isolates from foods and water (12, 27, 71), and for isolates included in the description of new *Arcobacter* species in order to find out whether they have a clonal origin (24, 28, 34, 74, 75). However, in the recent description of the new species *A. trophiarum*, 10 different AFLP profiles were recognized among the 16 isolates recovered from this species, while with ERIC-PCR only 4 genotypes could be identified (34). These differences are surprising since ERIC-PCR has been the recommended technique for genotyping *Arcobacter* strains on the basis of the results obtained in several studies (69, 71). This indicates that the resolution power of these typing techniques should be reevaluated for *Arcobacter* by sequencing those strains, since so far there are no comparative studies on this genus that provide evidence that AFLP has a better resolution than ERIC-PCR.

The first website database for multilocus sequence typing (MLST) (http://pubmlst.org/arcobacter/) has recently been created by Miller et al. (111), who analyzed seven genes of 374 strains belonging to five species of the genus (*A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. cibarius*, and *A. thereius*). The website provides information for primers and sequencing conditions for the seven genes (*aspA*, *atpA*, *glnA*, *gltA*, *pgm*, *tkt*, and *glyA*) and for submitting new sequences. The results of the MLST approach did not, however, find any association between the sequence types and the host or geographical sources, thus corroborating the high genetic diversity within the *Arcobacter* spp. reported in previous studies using other typing methods (12, 27, 71, 79, 118). The wide variation in the genotypes may be due to multiple sources of contamination (12) or, as has been suggested for *Campylobacter*, to their ability to incorporate exogenous DNA or to undergo genomic rearrangement by multiple recombinations (79), but this has not yet been demonstrated for *Arcobacter* species (27).

CONCLUSIONS AND PERSPECTIVES

It has been almost 2 decades since the aerobic and nonthermophilic genus *Arcobacter* was described for the first time from species previously included in *Campylobacter*. Despite a considerable amount of information having been accumulated in this period, knowledge remains far behind that for *Campylobacter*. In the last 5 years, the number of new species has risen exponentially due to the application of molecular techniques (m-PCR, 16S rRNA gene-RFLP, and sequencing of the 16S rRNA gene), as has the number of habitats from which they have been discovered (skins of poultry carcasses, a hypersaline lagoon, mussels, kidneys and livers of aborted pig fetuses, feces of piglets, cloacal contents of ducks and chickens, seawater, sewage, and oysters). At the same time, new phenotypic characters for the genus have been recognized, such as the halophilic property of *A. halophilus* and the inability to hydrolyze indoxyl acetate discovered for the species *A. mytili* (isolated from mussels) and also observed for the recently proposed new species *A. molluscorum* (isolated from the same type of samples and from oysters). The phylogenetic analysis of the 16S rRNA genes of sequences deposited at databases shows that there are still an important number of new species waiting to be described, many of which are unculturable bacteria, some of them from new environments (cyanobacterial mats, contaminated oil fields, coral, plankton, tubeworms, abalone, cod larviculture, snails, etc.). The ecological role that arcobacters may play in those environments is still not clear. Considering that fact and the data derived from the available complete genome of *A. butzleri*, the members of the genus have been classified as free-living, waterborne organisms that can be isolated from food and that can be emerging pathogens for humans and animals. In humans, arcobacters have been associated with bacteremia and diarrhea, while in animals there have been cases of diarrhea, abortion, and mastitis. Fecally contaminated water and food products (especially poultry and red meat, milk, and shellfish, which have often shown to be contaminated with arcobacters) have been suggested as the transmission routes, but this has not yet been demonstrated by identifying the same strain associated with the disease from food or water samples. To that end, the classical genotyping techniques applied to the genus (ERIC-PCR, RAPD-PCR, AFLP, and PFGE) and the recently developed MLST may be useful. It has been indicated that arcobacters are zoonotic agents and that livestock, i.e., poultry and pigs, can be reservoirs of arcobacters, and recent evidence seems to support their ability to colonize the poultry intestine. However, it is unknown whether wild birds may also play an epidemiological role, as occurs for *Campylobacter*. So far, only the species *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus* have been recovered from human and animal infections. This has happened on several occasions and has been associated with aborted bovine or porcine fetuses. The recently described species *A. thereius* was also recovered from livers and kidneys of spontaneous porcine abortions, but the pathogenic role of this and other species has not yet been established. So far, only one study has investigated and demonstrated the dissemination to the fetus in a case of an infection in sows, but the specific pathogenic mechanisms of *Arcobacter* spp. in animal reproduction abnormalities remain unknown. There is a similar lack of knowledge in the case of mastitis, where the infection has also been reproduced. Therefore, Koch's classical postulates have been partially fulfilled in these examples and in others (for diarrhea and fish infection). Data on the association between diarrhea and *Arcobacter* in humans have accumulated, with various incidences, and have so far involved the three mentioned species, while the scattered cases of bacteremia has never involved *A. skirrowii*. Very recently, *A. butzleri* was considered the etiological agent of traveler's diarrhea for the first time. Furthermore, an experimental study using strains of that species on human colonic epithelial cells have revealed their capacity to produce a barrier dysfunction leading to a leak flux type of diarrhea. Despite the increasing accumulated evidence, the true enteropathogenicity (association versus causation) has not been fully demonstrated in human volunteers. For instance, nothing is known about whether the interaction between the bacteria and the host generates an immunological response. More epidemiological evidence is needed to support existing knowledge and to be able to answer important questions. Studies that fully characterize the products involved in the adhesion, invasion, and cytotoxicity observed in different cell lines are in their infancy, and there is a need to discern these processes at the molecular level, elucidating the receptors of adhesion, the role of flagella, the effectors needed for invasions, etc. Despite putative virulence genes homologous to those of the *C. jejuni* genome being recognized in the *A. butzleri* genome, their functionality remains to be proven, as well as what the host circumstances are and which genes are required to develop disease. Generation of mutants and novel developments in animal models may help to answer these questions.

There is a need for clinicians to be aware of the possible role that arcobacters may play in human and animal disease. An effort should be made to use culturing, detection, and identification techniques that allow the recovery and identification of all recognized *Arcobacter* species in order to know their true implication in human and veterinary medicine, as well as their prevalence in environmental samples (food, water, etc.). Regarding that, and awaiting improved procedures, it is advisable to use an enrichment step in a broth (CAT, etc.) followed by passive filtration of the broth (0.45-μm filters) on blood agar (both incubated at 37°C for 48 to 72 h) and molecular identification of as many colonies as possible in parallel to direct detection by PCR.

The newly available complete genome from *A. nitrofigilis* needs to be fully analyzed by comparative genomics with the available one from *A. butzleri*. This will give new insights into the taxonomic position of the genus, its adaptation to the environment or the host, and its virulence potential and pathogenicity. This information will be complemented in the near future by the new ongoing or future genome sequencing projects. The application of already-available microarray and proteomic analyses may provide important additional information. It can be expected that in the coming years we will witness remarkable changes in the understanding of these microbes.

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