




Review

# A Review on the Prevalence of *Arcobacter* in Aquatic Environments

Rajani Ghaju Shrestha <sup>1,2</sup> , Yasuhiro Tanaka <sup>3</sup>  and Eiji Haramoto <sup>1,\*</sup> 

<sup>1</sup> Interdisciplinary Center for River Basin Environment, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511, Yamanashi, Japan; rajanighaju@gmail.com

<sup>2</sup> Division of Sustainable Energy and Environmental Engineering, Osaka University, 1-1 Yamadaoka, Suita 565-0871, Osaka, Japan

<sup>3</sup> Department of Environmental Sciences, University of Yamanashi, 4-4-37 Takeda, Kofu 400-8510, Yamanashi, Japan; yasuihiro@yamanashi.ac.jp

\* Correspondence: eharamoto@yamanashi.ac.jp

**Abstract:** *Arcobacter* is an emerging pathogen that is associated with human and animal diseases. Since its first introduction in 1991, 33 *Arcobacter* species have been identified. Studies have reported that with the presence of *Arcobacter* in environmental water bodies, animals, and humans, a possibility of its transmission via water and food makes it a potential waterborne and foodborne pathogen. Therefore, this review article focuses on the general characteristics of *Arcobacter*, including its pathogenicity, antimicrobial resistance, methods of detection by cultivation and molecular techniques, and its presence in water, fecal samples, and animal products worldwide. These detection methods include conventional culture methods, and rapid and accurate *Arcobacter* identification at the species level, using quantitative polymerase chain reaction (qPCR) and multiplex PCR. *Arcobacter* has been identified worldwide from feces of various hosts, such as humans, cattle, pigs, sheep, horses, dogs, poultry, and swine, and also from meat, dairy products, carcasses, buccal cavity, and cloacal swabs. Furthermore, *Arcobacter* has been detected in groundwater, river water, wastewater (influent and effluent), canals, treated drinking water, spring water, and seawater. Hence, we propose that understanding the prevalence of *Arcobacter* in environmental water and fecal-source samples and its infection of humans and animals will contribute to a better strategy to control and prevent the survival and growth of the bacteria.

**Keywords:** *Arcobacter*; fecal-source samples; public health; water



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## 1. Introduction

The genus *Arcobacter* is a gram-negative, microaerophilic, nonspore-forming, motile, and spiral-shaped bacterium classified under the family *Arcobacteraceae* (formerly classified under *Campylobacteriaceae*) [1]. *Arcobacter* was first isolated in 1977 from bovine and pig fetuses in Belfast, UK [2]. Since then, 33 species have been recognized (Table 1), and different physiological characteristics have been identified (Table 2). Furthermore, these *Arcobacter* species have been isolated from humans, animals, natural and marine water environments, sewage, and septic tanks worldwide.

*Arcobacter* causes various diseases, such as livestock reproductive problems, mastitis, and gastric ulcers in animals, including gastroenteritis, bacteremia, peritonitis, and endocarditis in humans [3]. Furthermore, following *Arcobacter* infections (diarrhea and bacteremia), severe cases in humans have mainly been caused by *A. butzleri*, followed by *A. cryaerophilus*, whereas *A. skirrowii* and *A. thereius* have only been rarely reported [4]. Although the predominant transmission route is via contaminated foods, untreated water samples have also been recognized as a potential source of infection [5].

Among various *Arcobacter* spp., *A. butzleri* is one of the enteric waterborne bacterial pathogens to be considered when managing community drinking water risks [6]. During

the three reported waterborne outbreaks associated with *Arcobacter* that occurred in Finland, Slovenia, and the US, the bacterium was isolated either from drinking water or the feces of patients with diarrhea. In all cases, their drinking water was fecally contaminated [7–9]. Thus, it is vital to understand the correlation between *Arcobacter* presence in water, food sources, and fecal contamination. This review paper describes the prevalence of *Arcobacter* in water, food, and fecal samples to understand its association with fecal contamination in water and food products.

**Table 1.** List of *Arcobacter* species with their sources and countries of origin.

Species	Sources	Country	References
<i>A. acticola</i>	Seawater	Korea	[10]
<i>A. anaerophilus</i>	Estuarine sediment	India	[11]
<i>A. antarcticus</i>	Antartic intertidal sediment	Antartica	[12,13]
<i>A. aquimarinus</i>	Seawater	Spain	[14]
<i>A. arenosus</i>	Marine sediment	Korea	[13,15]
<i>A. bivalviorum</i>	Shellfish	Spain	[16]
<i>A. butzleri</i>	Feces (humans with diarrhea)	USA	[17]
<i>A. caeni</i>	Reclaimed water	Spain	[18]
<i>A. canalis</i>	Water canal	Spain	[19]
<i>A. cibarius</i>	Broiler carcass	Belgium	[20]
<i>A. cloacae</i>	Sewage	Spain	[21]
<i>A. cryaerophilus</i>	Animal abortions	Ireland	[1]
<i>A. defluorii</i>	Sewage	Chile	[22]
<i>A. ebronensis</i>	Mussels	Spain	[14]
<i>A. ellisii</i>	Mussels	Spain	[23]
<i>A. faecis</i>	Septic tank	Canada	[24]
<i>A. halophilus</i>	Hypersaline lagoon	USA	[25]
<i>A. lacus</i>	Reclaimed water	Spain	[18]
<i>A. lanthieri</i>	Pig and dairy cattle manure	Canada	[26]
<i>A. lekinthochrous</i>	<i>Pecten maximus</i> larvae and tank seawater	Norway	[27]
<i>A. marinus</i>	Dokdo island	Korea	[28]
<i>A. molluscorum</i>	Shellfish	Spain	[29]
<i>A. mytili</i>	Mussels	Spain	[30]
<i>A. nitrofigilis</i>	Roots of <i>Spartina alterniflora</i>	USA	[1]
<i>A. pacificus</i>	Seawater	China	[31]
<i>A. parvus</i>	Squid	Korea	[13,32]
<i>A. skirrowii</i>	Feces (humans with diarrhea)	USA	[17]
<i>A. suis</i>	Pork meat	Spain	[21]
<i>A. thereius</i>	Pigs and ducks	Belgium	[33]
<i>A. trophiarum</i>	Pigs	Belgium	[34]
<i>A. vandammei</i>	Porcine intestine	Belgium	[35]
<i>A. venerupis</i>	Shellfish	Spain	[16]
<i>A. vitoriensis</i>	Wastewater	Spain	[13,36]

**Table 2.** Differential physiological characteristics of all identified genus *Arcobacter* species.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			
Growth in/on:																																				
Air at 37 °C	–	–	–	+	+	+	+	–	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+	*	+	–	–	–	+	–	–	+		
CO <sub>2</sub> at 37 °C	*	–	*	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+	*	+	–	–	–	–	+	+	+		
CO <sub>2</sub> at 42 °C	*	–	*	–	*	–	+	–	+	–	–	–	+	–	+	–	–	+	–	*	–	+	+	–	–	*	–	–	–	–	–	–	–	–		
4% (w/v) NaCl	–	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–		
1% (w/v) Glycine	*	+	*	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	*	*	–	–	+	–	–	–	–	–		
MacConkey agar	*	*	+	–	*	+	–	+	V	+	+	–	–	–	+	+	–	+	+	–	+	+	+	–	–	+	–	+	–	–	+	*	–	+		
CCDA	*	*	–	+	*	+	+	+	–	–	+	+	+	–	+	+	–	+	+	–	–	–	–	–	*	*	+	–	–	+	*	+	+	+		
Enzyme activity:																																				
Urease	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–	*	–	–	–	–	–	–	+	*	
Catalase	+	–	*	+	–	+	+	+	+	–	+	+	–	–	+	+	–	+	+	+	–	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+
Esterase	–	*	*	*	*	+	+	*	*	+	*	+	–	*	+	+	+	+	*	+	–	+	+	+	+	–	–	+	+	*	–	–	–	–	–	+
Hippurate hydrolysis	*	*	*	*	*	–	–	*	*	–	*	–	–	*	+	–	–	*	–	*	–	–	–	–	*	+	–	*	–	*	–	–	*	*	*	
Alkaline phosphatase	*	*	*	*	*	+	+	*	*	–	*	+	–	*	–	–	–	*	–	–	+	+	+	–	–	+	–	*	–	*	–	–	*	*	–	
Voges-Proskauer test	*	*	*	*	*	–	–	*	*	–	*	–	–	*	–	+	–	*	+	–	+	–	–	–	*	*	–	*	–	*	–	–	*	*	*	+
Na-succinate assimilation	*	*	*	*	*	+	–	*	*	–	*	+	–	*	–	–	+	*	–	*	–	–	–	–	*	*	–	*	–	*	–	–	+	*	*	*
Nitrate reduction	–	+	*	+	–	–	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Nitrite production	–	*	*	*	*	–	–	*	*	–	*	+	–	*	–	–	–	*	+	*	–	–	–	–	–	*	*	–	*	–	–	–	*	*	*	*
TTC reduction	*	*	*	*	*	–	+	–	–	+	*	–	–	*	–	+	+	–	+	+	–	–	–	+	–	*	W	–	*	–	–	–	–	*	+	+
Indoxyl acetate hydrolysis	*	+	*	+	*	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	*
Resistance to cefoperazone (64 mg/L)	*	–	*	–	*	–	+	+	–	+	–	+	+	–	–	–	–	+	+	–	–	–	–	–	+	*	+	–	+	+	*	–	–	–	+	

Strains/species: 1, *A. acticola*; 2, *A. anaerophilus*; 3, *A. antarcticus*; 4, *A. aquimarinus*; 5, *A. arenosus*; 6, *A. bivalviorum*; 7, *A. butzleri*; 8, *A. caeni*; 9, *A. canalis*; 10, *A. cibarius*; 11, *A. cloacae*; 12, *A. cryaerophilus*; 13, *A. defluvi*; 14, *A. ebronensis*; 15, *A. ellisii*; 16, *A. faecis*; 17, *A. halophilus*; 18, *A. lacus*; 19, *A. lanthieri*; 20, *A. lekinthochrous*; 21, *A. marinus*; 22, *A. molluscorum*; 23, *A. mytili*; 24, *A. nitrofigilis*; 25, *A. pacificus*; 26, *A. parvus*; 27, *A. skirrowii*; 28, *A. suis*; 29, *A. thereius*; 30, *A. trophiarum*; 31, *A. vandammei*; 32, *A. venerupis*; 33, *A. vitoriensis*. Data for *Arcobacter* species were obtained from sources of [10–12,14,15,18,19,24,26,27,31,32,35,36]. “+”, Positive. “–”, Negative. “\*”, Not determined. V, 12–94% strains positive. W, weakly positive.

### 1.1. Pathogenicity of *Arcobacter*

The complete genome sequencing and analysis of *A. butzleri* revealed nine putative virulence genes (*cadF*, *cj1349*, *ciaB*, *mviN*, *pldA*, *tlyA*, *hecA*, *hecB*, and *irgA*) [37]. These genes have received particular attention due to their homology to genes associated with pathogenicity in other microorganisms [37]. For example, some virulence determinants identified in *Campylobacter jejuni* homologs within *A. butzleri* are fibronectin-binding proteins (*cadF* and *cj1349*), the invasion protein (*ciaB*), the virulence factor (*mviN*), the phospholipase (*pldA*), and hemolysin (*tlyA*). Other putative virulence determinants *irgA*, *hecA*, and *hecB* present in *A. butzleri* have also been identified in *Vibrio cholera*, uropathogenic *Escherichia coli*, *Erwinia crysthanthemii*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Burkholderia cepacia*, and *Acinetobacter* [37]. After developing a polymerase chain reaction (PCR) assay to detect those nine putative virulence genes, they were primarily present in *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* isolates [38]. Virulence genes have also been identified in *A. cibarius*, *A. trophiarum*, *A. defluvi*, *A. molluscorum*, *A. ellisii*, *A. bivalviorum*, *A. venerupis*, *A. suis*, *A. cloacae*, *A. faecis*, and *A. lanthieri*, but not in *A. thereius* and *A. mytili* [39,40].

The pathogenicity of these virulence genes was also determined based on the ability of the bacteria to adhere, invade, and produce toxins on human and animal cell lines [4]. Therefore, although *A. thereius* and *A. mytili* did not possess the virulence genes as stated above, they could adhere to and invade Caco-2 cell lines [39]. Hence, these species could have some public health importance, considering that they were identified in animals (porcine abortion) and mussels, respectively [30,33]. However, an unclear association between the presence of virulence genes and the pathogenicity associated with cell lines has been observed [4]. As all species of *Arcobacter* might not be pathogenic, such as nitrogen-fixing *A. nitrofigilis* [1], more studies should be conducted regarding useful aspects, pathogenicity and virulence potentials of the *Arcobacter* species.

### 1.2. Antimicrobial Resistance of *Arcobacter*

Antimicrobial susceptibility tests of *Arcobacter* isolated from water, food-related origins, and humans have been conducted [41]. In most of these studies, the susceptibility test has been limited to three species of *Arcobacter*: *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* because of the severity of their infections in humans and animals [3]. This antimicrobial susceptibility test was conducted through various methods, i.e., agar plate dilution, broth microdilution, disk diffusion, gradient strip diffusion, and Sensititre™ semiautomated [42]. Based on the treatment of *Arcobacter*-dependent infections, antibiotics used have included quinolones, cephalosporins, tetracyclines, macrolides, and  $\beta$ -lactam antibiotics combined with  $\beta$ -lactamase inhibitors. It has also been reported that *Arcobacter* was resistant to various classes of antibiotics, such as penicillins (69.3–99.2%), cephalosporins (30.5–97.4%), macrolides (10.7–39.8%), fluoroquinolones (4.3–14.0%), aminoglycosides (1.8–12.9%), and tetracyclines (0.8–7.1%). Furthermore, *A. butzleri* was more resistant to several antibiotics compared with other species of *Arcobacter* [42]. Therefore, the higher resistance rate of *A. butzleri* proposes that this species can act as a reservoir of genes, contributing to antimicrobial resistance dissemination through various mediums. However, the high prevalence of antimicrobial resistance can be because of *Arcobacter* spp. exposure to antibiotics used both in animal production and human medicine.

## 2. Methods for Detecting *Arcobacter*

### 2.1. Methods for Isolating and Cultivating *Arcobacter*

*Arcobacter* was first isolated from livestock abortions using an Ellinghausen–McCullough–Johnson–Harris (EMJH) *Leptospira* culture media [2]. Since then, various enrichment and isolation techniques have been used to isolate *Arcobacter* from different samples (Table 3). Therefore, isolating *Arcobacter* through culture mediums has been performed using enrichment media, such as EMJH, *Arcobacter* specific broth (ASB), Cefoperazone, amphotericin, teicoplanin (CAT) broth, Johnson–Murano broth (JMB), *Arcobacter* broth, and *Arcobacter* enrichment basal mediums supplemented with antibiotics have been used

to enrich *Arcobacter* from meats, ground pork, broiler chicken, and poultry products. These supplementation antibiotics include 5-fluorouracil, cefoperazone, piperacillin, trimethoprim, cycloheximide, amphotericin B, teicoplanin, and novobiocin [2,43–47] which help in the selective growth of the bacteria with no growth of competing microorganisms. The incubation conditions varied depending upon the types of broth, mostly at 30 °C for 48–72 h under aerobic or microaerophilic conditions. After enrichment, different plating media, including Cephalotin, vancomycin, the amphotericin B (CVA) agar, Johnson–Murano (JM) agar, modified charcoal cefoperazone deoxycholate agar (mCCDA), and the *Arcobacter* plating medium were used with antibiotics mentioned above in enrichment media and blood agar without antibiotics [2,43–46,48], after which they were incubated at various conditions mostly at 30 °C for 48–72 h under aerobic or microaerophilic conditions as shown in Table 3. Although these enrichment and isolation techniques were developed in the 2000s, they are still widely used to isolate *Arcobacter* from water, animal meat, milk, rectal swab, and floor swab samples [49–51]. Additionally, a selective enrichment broth and a selective-differential plating medium were developed for the growth of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* in food samples. The medium has 97.8% inclusivity for *Arcobacter* and 100% exclusivity for non-*Arcobacter* strains. [52]. As reported, the culture-dependent approach helps determine recovered isolates' antibiotic susceptibilities from clinically significant samples [53]. Some studies have also proposed that a reduction in the diversity of species because of the enrichment step can also affect their direct molecular detection from enrichment broth [50,54]. However, identifying *Arcobacter* isolates at the species level is difficult because of insufficient biochemical tests and difficulties in phenotypic characterization of the bacterium [55]. Therefore, molecular methods are convenient for the rapid and accurate identification of *Arcobacter* at the species level.

## 2.2. Molecular-Based Detection Methods of *Arcobacter*

With several drawbacks regarding the need for a rapid, reliable, and sensitive technique for the specific detection of *Arcobacter*, PCR assays were developed by Bastyn et al. [57] and Harmon and Wesley [58], targeting 23S and 16S rRNA genes of *Arcobacter*, respectively. Therefore, these assays have been used extensively as an alternative to conventional microbiological culture methods to identify *Arcobacter* from drinking and environmental water, including milk, meat, clams, mussels, cattle, and fecal samples (Table 4). Furthermore, since *A. butzleri*, *A. skirrowii*, and *A. cryerophilus* were associated with human and animal illness [33], a multiplex PCR assay targeting the 16S and 23S rRNA was developed for the detection and identification of these three *Arcobacter* species [59]. This assay was extensively applied in ponds, springs, seawater, river water, wastewater, drinking water, milk, chickens, dogs, cats, cattle, meat, and feces. In addition to the three above mentioned species of *Arcobacter*, Pentimalli et al., [60] developed species-specific primers, targeting *gyrA* and 16S rRNA gene sequences of *A. cibarius*, including three above mentioned species of *Arcobacter*, as *A. cibarius* was isolated from the skin of broiler chicken carcasses and piggery effluents [20,61]. Subsequently, a developed PCR assay was applied to survey *Arcobacter* contaminations in chicken meat. Later in 2010, Doudah et al. [62] developed a multiplex PCR assay, targeting five human- and mammal-associated *Arcobacter* spp., to examine 16S and 23S RNA, *rpoB*, and *gyrA* genes. This multiplex PCR assay identified *Arcobacter* species in raw milk from vending machines in Italy [63].

Table 3. Isolation and cultivation methods for *Arcobacter*.

Enrichment		Isolation		References
Formulation Antibiotics Used (mg/L)	Incubation Conditions	Plating Medium Antibiotics Used (mg/L)	Incubation Conditions	
EMJH 5-Fluorouracil (100) ASB	30 °C, 48–72 h, mO <sub>2</sub>	Blood agar No antibiotics ASM	30 °C, 48–72 h, mO <sub>2</sub> , and O <sub>2</sub>	[2]
Cefoperazone (32) Piperacillin (75) Trimethoprim (20) Cycloheximide (100)	24 °C, 48 h, O <sub>2</sub>	Cefoperazone (32), Piperacillin (75) Trimethoprim (20) Cycloheximide (100)	24 °C, 48–72 h, O <sub>2</sub>	[45]
EMJH 5-Fluorouracil (200)	30 °C, 9 days, O <sub>2</sub>	CVA agar Cephalothin (20) Vancomycin (10) Amphotericin B (5)	30 °C, up to 7 days, O <sub>2</sub>	[44]
CAT broth Cefoperazone (8) Amphotericin B (10) Teicoplanin (5)	30 °C, 48 h, mO <sub>2</sub>	Blood agar No antibiotics Membrane filtration	30 °C, up to 7 days, O <sub>2</sub>	[43]
JM broth Cefoperazone (16), 5-Fluorouracil (200)	30 °C, 48 h, O <sub>2</sub>	JM agar Cefoperazone (32)	30 °C, 48 h, O <sub>2</sub>	[46]
-	-	modified charcoal cefoperazone deoxycholate agar (mC- CDA)	37 °C, 48 h, mO <sub>2</sub>	[48]
Arcobacter broth Cefoperazone (16), Amphotericin B (10) 5-Fluorouracil (100) Novobiocin (32) Trimethoprim (64)	28 °C, 48 h, mO <sub>2</sub>	Cefoperazone (32) Arcobacter plating medium Cefoperazone (16), Amphotericin B (10) 5-Fluorouracil (100) Novobiocin (32) Trimethoprim (64)	30 °C, 24–72 h, mO <sub>2</sub>	[56]
Nguyen-Restaino-Juárez (NRJ) broth Cefsulodin (6), vancomycin (4), and moxalactam (10)	30 °C, 48 h, O <sub>2</sub>	NRJ medium cefsulodin (10), vancomycin (1), novobiocin (1), and moxalactam (10)	30 °C, 48 h, O <sub>2</sub>	[52]

EMJH, Ellinghausen–McCullough–Johnson–Harris semisolid medium; ASB, Arcobacter selective broth; CAT, Cefoperazone, amphotericin, teicoplanin broth; JM, Johnson–Murano; ASM, Arcobacter selective medium; CVA, cephalotin, vancomycin and amphotericin B agar; mO<sub>2</sub>, microaerobic conditions; O<sub>2</sub>, aerobic conditions.

In addition to these conventional PCR assays, qPCR has also been developed to evaluate their applicability to the newly discovered *Arcobacter* species with high specificity and sensitivity [64–67]. Similarly, fluorescent in situ hybridization (FISH) has been used to identify *Arcobacter* in river water and wastewater samples [49,68,69]. However, since fewer recent studies regarding pathogenicity, virulence genes, and the discovery of new *Arcobacter* species exist, assays targeting wide *Arcobacter* species should be developed. Additionally, the applicability of previously developed assays should be extended to monitor all newly discovered *Arcobacter* species.

**Table 4.** Specific detection of *Arcobacter* using molecular-based methods.

Methods	Genes Targeted	Species Identified	References
RFLP, Southern blotting	16S rRNA, 23S rRNA	<i>A. butzleri</i>	[70]
PCR-hybridization	<i>glyA</i>	<i>A. butzleri</i>	[71]
Real time PCR	<i>gyrA</i>	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. cibarius</i> , <i>A. nitrofigilis</i>	[72]
Real time PCR	<i>rpoBC</i> , 23S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[73]
Multiplex PCR	<i>rpoBC</i> , 23S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[73]
MALDI-TOF MS	Proteins	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i> ,	[74]
PCR	23S rDNA	<i>Arcobacter</i> spp.	[57]
Multiplex PCR	16S rRNA, 23S rRNA	<i>Arcobacter</i> spp., <i>A. butzleri</i>	[75]
PCR-RFLP	16S rRNA	<i>A. butzleri</i>	[76]
PCR-RFLP	23S rRNA	<i>A. butzleri</i> , <i>A. nitrofigilis</i>	[77]
In situ hybridization	16S rRNA	<i>Arcobacter</i> spp.	[69]
PCR-RFLP	16S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[78]
PCR-culture	16S rRNA	<i>Arcobacter</i> spp.	[65]
Multiplex PCR	16S rRNA, 23S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[59]
Multiplex PCR	23S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[79]
PCR-RFLP	<i>groEL</i>	<i>A. butzleri</i>	[80]
PCR-RFLP	16S rRNA, 23S rRNA	<i>A. butzleri</i>	[81]
PCR-DGGE	16S rRNA	<i>A. cryaerophilus</i> , <i>A.</i> <i>nitrofigilis</i>	[82]
PCR-RFLP	16S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i> , <i>A. cibarius</i> , <i>A.</i> <i>nitrofigilis</i> , <i>A. halophilus</i> , <i>A.</i> <i>cibarius</i> , <i>A. mytili</i>	[83]
PCR	<i>gyrA</i> , 16S rRNA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i> , <i>A. cibarius</i>	[60]
Multiplex PCR	23S rRNA, <i>gyrA</i>	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i> , <i>A. cibarius</i> , <i>A.</i> <i>thereius</i>	[62]
PCR	<i>hsp60</i>	<i>A. trophiarum</i>	[34]
PCR	16S rRNA	<i>Arcobacter</i> spp.	[66]
PCR	16S rRNA	<i>A. butzleri</i>	[84]
MPN-qPCR	<i>hsp60</i>	<i>Arcobacter</i> spp.	[85]
qPCR	16S rRNA	<i>Arcobacter</i> spp.	[64]

RFLP, restriction fragment length polymorphism; DGGE, denaturing gradient gel electrophoresis; MALDI-TOF MS, matrix-associated laser desorption ionization-time-of-flight mass spectrometry; MPN-qPCR, most probable number-qPCR.

### 3. The Specific Detection of *Arcobacter* in Animal Feces and Products

Although most *Arcobacter* species are reported as commensal in the gastrointestinal tract of animals, the feces of animals are considered a possible source of contamination [50]. Therefore, transmission routes are regarded as fecally contaminated food and water [51,86]. As summarized in Table 5, *Arcobacter* has been detected in various fecal samples of cows, dogs, cats, pigs, chickens, and their products [5,84,87–90]. Methods applied for detecting *Arcobacter* in these samples include culture and PCR, whereas methods applied for typing *Arcobacter* include enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), multilocus sequence typing (MLST), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Table 4). Moreover, studies have reported that the positive percentage of *Arcobacter* detected in fecal samples from humans and animals ranged from 3% to 60%. Additionally, the discharge of feces from farms with cows, pigs, horses, and sheep contains millions of bacterial cells [91]. Therefore, when discharged into the water environment, fecal pathogens serve as a potential source/reservoir of waterborne diseases and can be transferred via the food process chain and water samples.

*Arcobacter* has also been detected in human stool samples. In a study by Webb et al., *Arcobacter* was identified in the stool of diarrheic and non-diarrheic people [84]. The study identified no difference in the prevalence of *Arcobacter* between diarrheic and non-diarrheic stools. However, in a research study conducted by Pérez-Cataluña et al., *Arcobacter* detected from clinical samples contained different virulence genes, resistant to antibiotics [92]. Additionally, in Belgium and France, *Arcobacter* is the fourth most common bacteria isolated from patients with enteric diseases' feces and the third most prevalent in South Africa [93].

The contamination of food with *Arcobacter* may result from improper hygienic practices at different stages of the food supply chain. Although the prevalence of *Arcobacter* in animal products varies greatly among different studies, it is found that poultry meat has a higher *Arcobacter* contamination ratio in comparison to red meat, raw cow milk and vegetables. In a study by Uljanovas et al., the highest contamination of *Arcobacter* was observed in chicken meat followed by raw milk [94]. In a study conducted by Travesera et al., milk samples intended for human consumption sold through vending machines were found positive for *Arcobacter* [63]. This shows that poor handling or consumption of contaminated meat or raw milk may cause adverse effects on human health. Therefore, *Arcobacter* might be one of the etiological factors for human gastroenteritis.



**Table 5.** Various samples from which *Arcobacter* was detected and isolated.

Sample Type	No. of Positive Samples/No. of Samples Tested (%)	Countries	<i>Arcobacter</i> spp. Identified	References	
Fecal samples	Human stool	Germany	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. lanthieri</i>	[5]	
		Canada	<i>A. butzleri</i>	[84]	
		Lithuania	<i>A. butzleri</i>	[94]	
	Cattle feces	20/51 (39)	Belgium	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[95]
		12/332 (4)	Japan	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[89]
		14/200 (7)	Turkey	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[96]
		240/1682 (14)	USA	<i>Arcobacter</i> spp.	[90]
	Pig feces	36/82 (44)	Belgium	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[95]
		25/250 (10)	Japan	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[89]
	Sheep feces	10/62 (16)	Belgium	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[95]
Horse feces	2/13 (15)	Belgium	<i>A. butzleri</i>	[95]	
Dog feces	5/267 (2)	Belgium	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[88]	
Cat feces	0/61 (0)	Belgium	Not detected	[88]	
Animal products	Beef meat	Turkey	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[53]	
		39/148 (26)	Malaysia	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[51]
		13/45 (29)	USA	<i>A. butzleri</i> , <i>A. skirrowii</i>	[97]
		37/108 (34)	Northern Ireland	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[98]
		7/32 (22)	Australia	<i>A. butzleri</i>	[99]
		2/90 (2)	Japan	<i>A. butzleri</i>	[100]
	5/97 (5)	Turkey	<i>A. butzleri</i>	[96]	
	1/68 (1)	The Netherlands	<i>Arcobacter</i> spp.	[45]	
	Pork meat	7/100 (7)	Japan	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[100]
		64/200 (32)	USA	<i>Arcobacter</i> spp.	[101]
		1/27 (4)	Italy	<i>A. butzleri</i>	[102]
		35/101 (35)	Northern Ireland	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[98]
		23/45 (51)	USA	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[97]
	Sheep meat	2/13 (15)	Australia	<i>A. butzleri</i>	[99]
	Chicken livers and carcasses	29/32 (91)	Spain	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[87]
89/170 (52)		Germany	<i>A. butzleri</i>	[103]	
Chicken meat	30/51 (59)	Japan and Thailand	<i>A. butzleri</i>	[104]	
	36/42 (86)	Spain	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[60]	
	53/220 (24)	The Netherlands	<i>Arcobacter</i> spp.	[45]	
	23/100 (23)	Japan	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[100]	
	6/15 (40)	USA	<i>A. butzleri</i> , <i>A. skirrowii</i>	[97]	
	16/22 (73)	Australia	<i>A. butzleri</i>	[99]	
	58/94 (62)	Northern Ireland	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[98]	
	119/331 (36)	Lithuania	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[94]	

Table 5. Cont.

Sample Type	No. of Positive Samples/No. of Samples Tested (%)	Countries	<i>Arcobacter</i> spp. Identified	References
Turkey meat	303/395 (77)	USA	<i>A. butzleri</i> , <i>Arcobacter</i> spp.	[105]
Duck carcass	8/10 (80)	UK	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[106]
Buccal cavity of dogs	2/267 (0.8)	Belgium	<i>A. cryaerophilus</i>	[88]
Buccal cavity of cats	0/61 (0)	Belgium	Not detected	[88]
Chicken cloacal swabs	34/234 (15)	Japan	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[89]
Cow's rectal swabs	8/120 (7)	Malaysia	<i>A. butzleri</i> , <i>A. skirrowii</i>	[51]
Cow milk	64/484 (13)	Italy	<i>A. butzleri</i>	[107]
	6/105 (6)	Malaysia	<i>A. butzleri</i> , <i>A. cryaerophilus</i> , <i>A. skirrowii</i>	[51]
	8/37 (22)	Italy	<i>A. butzleri</i>	[63]
	26/104 (25)	Lithuania	<i>A. butzleri</i>	[94]
Surface water	13/25 (52)	Czech Republic	<i>A. butzleri</i>	[108]
	10/10 (100)	Spain	<i>Arcobacter</i> spp.	[68]
	4/17 (24)	Japan	<i>A. butzleri</i>	[104]
	14/18 (78)	Nepal	<i>Arcobacter</i> spp.	[109]
	36/128 (28)	Lithuania	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[94]
Wastewater (Influent and effluent water)	9/9 (100)	UK	<i>A. butzleri</i> , <i>A. cryaerophilus</i>	[110]
	44/44 (100)	Spain	<i>Arcobacter</i> spp.	[111]
	30/30 (100)	Spain	<i>Arcobacter</i> spp.	[68]
	43/48 (90)	US	<i>Arcobacter</i> spp.	[112]
	29/30 (97)	Chile	<i>A. butzleri</i> , <i>A. cloacae</i> , <i>A. cryaerophilus</i> , <i>A. defluvi</i> , <i>A. ellisii</i> , <i>A. nitrofigilis</i> , <i>A. skirrowii</i> , <i>A. thereius</i>	[113]
	61/88 (69)	Italy	<i>A. butzleri</i>	[114]
	5/50 (10)	Italy	<i>Arcobacter</i> spp.	[115]
Canal	7/7 (100)	Japan	<i>A. butzleri</i>	[104]
Treated drinking water	2/18 (11)	Malaysia	<i>A. butzleri</i> , <i>A. skirrowii</i>	[51]
Sewage	24/66 (36)	Czech Republic	<i>A. butzleri</i>	[108]
Spring water	4/16 (25)	Czech Republic	<i>A. butzleri</i> , <i>Arcobacter</i> spp.	[108]
Drinking water	0/8 (0)	Czech Republic	Not detected	[108]
Groundwater	13/47 (26)	Nepal	<i>Arcobacter</i> spp.	[64]
	99/286 (35)	Nepal	<i>Arcobacter</i> spp.	[116]

#### 4. Specific Detection of *Arcobacter* in Water Samples

Water quality and human health are interrelated, as water is one of the possible bacterial transmission routes to humans and animals [3,50]. As shown in Table 5, *Arcobacter* has been detected in various water samples, such as groundwater, river water, wastewater, canal water, seawater, spring water, and drinking water. In this review, the quantitative data on *Arcobacter* showed its highest detection in sewage, with a detection percentage of >69%. Although the prevalence of *Arcobacter* in water samples might vary depending upon different studies, it is found that the *Arcobacter* detection rate was approximately 100% in most wastewater samples in tested countries, such as Italy, Spain, the UK, and the US. Nevertheless, wastewater treatment plants (WWTPs) have been developed that collect and treat wastewater. Subsequently, treated water is returned to the environment for irrigation and recreational purposes [117]. Therefore, WWTPs are unsurprisingly considered hotspots for the presence of *Arcobacter* since it has been detected in multiple countries through culture, qPCR, multiplex PCR, and FISH [68,110–114]. The presence of *Arcobacter* in both influent and effluent samples of WWTPs indicates the high tolerance capability of this bacteria, ultimately leading to persistence and spread. Since WWTPs are considered hotspots for the spread of antibiotic resistance genes [118], these genes might transfer to *Arcobacter* conducting to unsuccessful treatments of severe infections. As observed, in groundwater and river water, the positive percentage of *Arcobacter* ranged from 26% to 78% and 24% to 100%, respectively. However, in canals, seawater, springs, and drinking water, the positive percentage of *Arcobacter* was 100%, 36%, 25%, and 0%, respectively. Therefore, these results further indicate environmental water samples as popular sources for *Arcobacter*, which, if untreated and consumed directly or indirectly, is proposed to affect human and animal health.

The presence of *Arcobacter* in water samples has also been associated with waterborne outbreaks. These outbreaks occurred in Finland, Slovenia, and the US, where people experiencing acute gastroenteritis consumed water contaminated with *Arcobacter* [7–9,119]. The presence of *Arcobacter* in freshwater could be a potential source for the spread of infections to humans and animals as these bacteria can be consumed directly when such contaminated water is used for washing raw vegetables or indirectly by using river water for irrigation purposes or preparing foods for an animal without any treatment of *Arcobacter* contaminated water. Similarly, a study showed that *Arcobacter* could survive at different temperatures and in non-chlorinated drinking water for up to 16 days [119–121], showing that water sources can act as a reservoir and potential source of *Arcobacter* contamination to humans and animals. It also indicates the potential of *Arcobacter* as a waterborne pathogen. Nevertheless, another study reported that water samples treated with chlorination did not contain *Arcobacter* [119], suggesting the importance of chlorinating drinking water before use.

#### 5. Microbial Community Analyses of *Arcobacter* in Various Samples

High-throughput sequencing technology has provided a powerful approach to improving our understanding of microbial ecology in various environments [122]. Thus, culture-independent, high-throughput sequencing of the 16S rRNA gene fragment has successfully identified *Arcobacter* in sediments receiving wastewater effluents, river feedback, wastewater, and groundwater samples [64,109,112,116,123–129]. *Arcobacter* was also one of the dominant pathogenic bacteria identified in groundwater, river water, and wastewater samples. Furthermore, in a study conducted by Sigala and Unc, antibiotic-resistant *Arcobacter* was identified through pyrosequencing [130]. Therefore, the dominance of *Arcobacter* among pathogenic bacterial communities illustrates the persistence of these bacteria in the environment, highlighting the importance of detecting these bacteria in other food and fecal-source samples.

## 6. Conclusions

This review summarizes the general characteristics, pathogenicity, and methods of detecting *Arcobacter*. It also shows the presence of *Arcobacter* in various samples worldwide, including its persistence in the environment. *Arcobacter* has been detected in several water bodies and other animal feces, including animal products. The inter-relationship between water quality, human health, and fecal-source samples shows that *Arcobacter* plays an essential role in these three parameters. Furthermore, *Arcobacter* in water and fecal samples can be regarded as a potential risk for human and animal health. Therefore, it is necessary to identify and treat the bacteria present in environmental samples to decrease the risk of exposure and reduce their effects on humans and animals. Data concerning the potential relationship between *Arcobacter* in fecal and water samples remain unavailable, but such data are necessary to understand the potential risks of waterborne pathogens better. Thus, a more extensive and rigorous surveillance system should be implemented to obtain these data. Additionally, studies providing molecular epidemiology and reliable risk assessments of *Arcobacter* infections in humans should be conducted.

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