GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

ARCOBACTER

Graham Banting
University of Alberta
Edmonton, Canada

Maria José Figueras Salvat
Universitat Rovira i Virgili, IISPV
Tarragona, Spain

Citation:
Banting, G. and Figueras Salvat, MJ. (2017). Arcobacter. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). (A. Pruden, N. Ashbolt and J. Miller (eds), Part 3: Specific Excreted Pathogens: Environmental and Epidemiology Aspects - Section 2: Bacteria), Michigan State University, E. Lansing, MI, UNESCO. https://doi.org/10.14321/waterpathogens.22

Acknowledgements: K.R.L. Young, Project Design editor; Website Design: Agroknow (http://www.agroknow.com)

Last published: May 25, 2017

Summary

The genus *Arcobacter* was created in 1991 to accommodate two aerotolerant *Campylobacter* species and since then has rapidly evolved. As of 2017, the genus constitutes >22 species (with several more in process of description), with some described species being able to produce human bacteremia and diarrhea. *Arcobacter butzleri* has been the most prevalent species in meat products (chicken, pork, beef, lamb), milk, cheese and shellfish. This has led to the inclusion of *A. butzleri* in the list of microbes considered a serious hazard to human health by the International Commission on Microbiological Specifications for Foods. Nevertheless other species like *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, *Arcobacter thereius* and *Arcobacter tropiarum* have also been associated with disease in human and animals. In humans *Arcobacter* can produce bacteremia and isolation rates from patients with diarrhea range from 0.11% to 1.31%. Contaminated food or water is considered the likely route of transmission to human and animals. In three reported waterborne outbreaks, *Arcobacter* was recovered either from the drinking water or from the faces of the patients with diarrhea, and in all cases the drinking water was fecally contaminated. A clear correlation between the concentration of fecal indicator bacteria in the water and the presence of *Arcobacter* has been demonstrated and the persistence of these bacteria in wastewater indicates that this could be one ecological reservoir. Metagenomic studies have revealed a high prevalence (5 to 11%) and genetic diversity of arcobacters among the bacteria communities present in wastewater, and this has been associated with the growth ability of these bacteria within the sewage system. The dominant species in wastewater is *A. cryaerophilus* based on metagenomics, yet in studies that utilize culture enrichment *A. butzleri* is the prevailing species, suggesting a culture bias towards *A. butzleri*. Due to the requirement of selective culture to isolate *Arcobacter*, there may be an ascertainment bias regarding the true rates and species composition of *Arcobacter* from various environments including human stools. Conventional wastewater secondary treatment is not able to fully remove the bacteria of this genus but chlorination and UV treatment seems to be effective. Currently, *Campylobacter* spp. are seen as a suitable surrogate for treatment efficacy of arcobacters. However, caution should be taken as *Arcobacter* spp. are found at levels up to 4 $\log_{10}$ higher in raw wastewater than *Campylobacter* spp. and likely have the ability to replicate in wastewater. As *Arcobacter* is a relatively new genus, gaps exist in the understanding of its true prevalence, the genetics of its virulence and its infectious dose in humans. Therefore, the risks associated with exposure to *Arcobacter* spp. are still unclear.

Arcobacter

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of the Disease

The true burden of *Arcobacter* infection is currently unknown. The first cases date back to 1992 when an outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* was described in an Italian school (Vandamme et al., 1992b). Since then relatively few studies have been published investigating the presence of *Arcobacter* in humans (reviewed in Collado and Figueras, 2011; Figueras et al., 2014; Arguello et al., 2015). Evidence exists to suggest that *Arcobacter* induces gastroenteritis, however the biological nature of its enteropathogenicity has yet to be clearly determined (reviewed in Collado and Figueras, 2011). Reporting of this disease agent is limited and it will be some time before the full impact of *Arcobacter* infections on human health is known.

1.1.1 Global distribution

Arcobacter distribution in humans is currently unknown due to the emerging nature of reporting. However, it will likely have a broader distribution than *Campylobacter* due to its ability to survive and possibly replicate in water. Thus far the limited reported human cases have come from four continents, suggesting broad distribution. The information derived from culturing human stools from patients with diarrhea indicate a prevalence of *Arcobacter* ranging from 0.1% to 1.25%, while in studies that detected these bacteria in feces by PCR results were much higher ranging from 0.4% to 56.7% (Figueras et al., 2014; de Boer et al., 2013; Collado et al., 2013; Ferreira et al., 2014; Webb et al., 2016a). In a dedicated retrospective study, performed in Belgium from 2008 to 2013, *Arcobacter* spp. were the fourth most prevalent bacterial pathogen detected, with an incidence of 1.31% after studying 6,774 fecal samples from patients with enteritis (Van den Abeele et al., 2014). In various other studies, detection of *Arcobacter* by molecular methods showed vastly different rates of prevalence as compared to detection by culture, but the highest difference have been detected in a recent study ca. 60% vs. 0.8%, respectively (Webb et al. 2016a; and references therein). The introduction of MALDI-TOF as a fast identification tool in clinical laboratories will likely help with the future identification of additional clinical cases of *Arcobacter* infection (Figueras et al., 2014). It should be noted that culture enrichment using selective antibiotics might result in significant bias and underestimation of *Arcobacter* spp. other than *A. butzleri*. This is due to the fact that the antibiotics used in culture enrichment select for antibiotic-resistant strains, while preventing recovery of antibiotic-sensitive strains that may be of human health concern.

1.1.2 Symptomatology

The majority of cases of enteritis and bacteremia caused by *Arcobacter* appear to be self-limiting and do not require antimicrobial treatment. However, the severity or prolongation of symptoms may justify the use of antibiotic treatment being fluoroquinolones and tetracycline suggested for the treatment of human and animal infections.

1.2 Taxonomic Classification of the Agent(s)

The genus *Arcobacter* along with the closely related genus *Campylobacter,* are members of the family *Campylobacteraceae*. *Helicobacter* is another closely related genus, within the family *Helicobacteraceae*. Both *Campylobacteraceae* and *Helicobacteraceae* are members of the order Campylobacterales. *Arcobacter* is a relatively new genus, being proposed in 1991 to accommodate two aerotolerant species previously considered atypical campylobacters (Vandamme et al., 1991). Since then the number of described species has increased progressively from six species in 2008 to more than twenty-two species as of 2017 (http://www.bacterio.net/arcobacter.html). The majority of species described to date have been isolated from shellfish, livestock, marine environments, and sewage. *Arcobacter* differ from *Campylobacter* by their aerotolerance and ability to grow at lower temperatures (15°C). Only four species have been identified in humans to date: *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius*. Of these, *A. butzleri* and *A. cryaerophilus*, are likely the most clinically significant on the basis of the detection frequencies in humans to date.

1.2.1 Physical description of *Arcobacter* species

Arcobacter spp. are similar in appearance to *Campylobacter* spp.: Gram-negative, non-spore forming curved or helical rod shaped cells that are 0.2 to 0.9 x 1 to 3 microns (Vandamme et al., 1992a). They are motile via a single polar flagellum and move in corkscrew like motion. Growth requirements are somewhat less fastidious than for *Campylobacter* as most *Arcobacter* spp. display aerotolerance of at least 5% O_2 and a broad range of temperature tolerance (15°C to 42°C) depending upon species and/or strain. It is considered that the bacteria of this genus have the ability to grow from 15°C to 37°C with optimal growth in microaerobic conditions at 30°C. One recently described species, *A. anaerophilus*, has been shown to be an obligate anaerobe (Sasi Jyothsna et al., 2013), but this is atypical for the genus. An additional atypical member of the genus, *A. halophilus*, is an obligate halophile that requires at least 2% NaCl in media to grow (Donachie et al., 2005). Recently, *A. halophilus* and *A. marinus* were recovered from water and shellfish using *Arcobacter*-CAT enrichment liquid medium supplemented with 2.5% NaCl (w/v) followed by culturing on Marine Agar (Salas-Massó et al., 2016). The introduction of this protocol has allowed the identification of 40% more *Arcobacter*-positive samples from seawater than the conventional method without the salt supplement (Salas-Massó et al., 2016).

Of the species tested, *A. butzleri* appears to be the least fastidious showing growth on lactose, glucose and citrate with some strains displaying thermotolerance at 42°C. *A. butzleri* also displays tolerance to 1.5% NaCl and has the ability to reduce nitrate (Vandamme et al., 1992a; Levican et al., 2013a). The fast growing characteristics of *A. butzleri* in enrichment culture may mask the abundance of other species (as well as when enriching for campylobacters (Banting et al., 2016) such as *A. cryaerophilus* (Houf et al., 2002; Collado and Figueras, 2011; Levican, 2016; Levican et al., 2016). *A. cryaerophilus* was not recovered using medium supplemented with NaCl in the study of Salas-Massó et al. (2016), despite being the second most abundant species using the *Arcobacter*-CAT medium without salt, indicating that *A. cryaerophilus* cannot tolerate 2.5% NaCl. Therefore, the range of species recovered from the environment may depend upon the culture media and incubation conditions employed.

1.2.2 New variants

At least twelve new *Arcobacter* species have been described since 2009, making *Arcobacter* a rapidly expanding genus, due to the introduction of molecular identification methods. A further expansion of the genus is likely as these new methods are more routinely used. Some species like *A. cloacae* and *A. defluvii* have been isolated from sewage (Levican et al., 2013a) and recently *A. faecalis* and *A. lanthieri* have been recovered from a human waste septic tank and from pig and dairy cattle manure, respectively (Whiteduck-Léveillée et al., 2015a; 2015b). Others have been isolated from marine shellfish: *A. bivalviorum* (Levican et al., 2012), *A. ebronensis* (Levican et al., 2015), *A. ellisii* (Figueras et al., 2011a), *A. molluscorum* (Figueras et al., 2011b), *A. mytili* (Collado et al., 2009a), *A. venerupis* (Levican et al., 2012) and *A. lekithochrous* (Diéguez et al., 2017). From seawater, *A. pacificus* (Zhang et al., 2015) and *A. aquimarinus* (Levican et al., 2015) have recently been described. There will likely be further species described, as specific culture methods are refined to enable efficient culture of novel *Arcobacter*s. In fact, the use of Arcobacter-CAT broth supplemented with 2.5% NaCl (w/v) with a subsequent culture on marine agar after passive filtration enabled the recovery of 52 new *Arcobacter marinus* and 6 *Arcobacter halophilus* isolates. These two species had previously only been known from single strains recovered the former from a mix of seaweeds, starfish and seawater from Korea and the latter from a hypersaline lagoon in Hawaii (Salas-Massó et al., 2016). The same approaches enabled recovery of several new species from shellfish and water, which have not yet been described (Salas-Massó et al., 2016). Additionally, a separate new species (*A. porcinus*) has been identified amongst strains included in the description of *A. thereius* (Figueras et al., 2017).

It is probable that the *Arcobacter* genus will be subdivided in the near future into two or more genera on the basis of: (1) 16S rRNA genetic differences between some type strains (<95%) and (2) their variable ecological niches (Figueras unpublished results).

1.3 Transmission

1.3.1 Routes of transmission

Formal transmission linkages are currently speculative, but based on knowledge of *Campylobacter* it seems that likely sources for *Arcobacter* transmission are fecal contamination combined with food and water matrices. Recreational water and drinking water are both likely sources of exposure along with irrigation water, as

Arcobacter appear to survive well in water (Van Driessche and Houf, 2008). Irrigation or washing of crops meant for raw consumption (vegetables) with water containing *Arcobacter* is a potential transmission route (Hausdorf et al., 2013; Fernandez-Cassi et al., 2016). *Arcobacter* has been found not only in raw food products but also in meals at popular restaurants in Bangkok where it was determined that the risk of exposure per consumed meal was 13%, and it was up to 75% in the case of 10 meals or more (Collado and Figueras, 2011; and references therein). *Arcobacter*, like *Campylobacter*, are also commonly found on poultry carcasses (Atanassova et al., 2008; Fallas-Padilla et al., 2014) and consumption of raw or undercooked poultry is likely a significant source of transmission. There is currently limited data on foodborne and waterborne *Arcobacter* transmission to determine its true importance in public health, as reviewed by Collado and Figueras (2011) and Hsu and Lee (2015).

1.3.2 Reservoirs

1.3.2.1 Human

There is limited data on human carriage rates of Arcobacter as only since the early 2000's have reports emerged studying Arcobacter in humans (Table 1). Historically, arcobacters were isolated from human feces as non-campylobacters via Campylobacter isolation protocols. However, the majority of Campylobacter isolation methods utilize a 42°C incubation temperature that is unlikely to be optimal for the majority of *Arcobacter* species/strains (Merga et al., 2011). Several large studies from the last decade have begun to shed light on *Arcobacter* in humans, but methods are not consistent between studies so caution must be used when interpreting results (Table 1). Two large studies from Belgium with a combined sample size of >47,000 patients have reported *Arcobacter* isolation from human feces by culture at rates ranging from 0.2%(Vandenberg et al., 2004) to 1.3% (Van den Abeele et al., 2014). In each study *Arcobacter* was detected at a frequency of 5% to10% to that of *Campylobacter* spp., with *A. butzleri*, *A. cryaerophilus* and *A. thereius* being the only species isolated. In each study, stools were collected from hospital patients who displayed some illness, which prompted a stool sample analysis. It was not determined if *Arcobacter* carriage was related to symptoms in either of these studies. A major caveat to most human carriage studies is that they rely upon culture enrichment followed by colony isolation on agar. It is likely that carriage rates are higher due to the inability to culture certain strains/species. This phenomenon has been clearly observed when using molecular detection methods. Other clinical studies have been reviewed (Figueras et al., 2014) and are in agreement with the frequency of detection in the large Belgian studies described above.

Table 1. Reported incidences of Arcobacter spp. detection in human stools

Area	Study Period	Species	Matrix	Method	Patient Age (y)	Percent Positive % (No. of Samples)	Reference
Belgium	1995 to 2002	*A. butzleri, A. cryaerophilus*	submitted for bacteriological stool culture	Direct plating on selective agar, SDS PAGE[a]	0 to 90	0.2% (77/40,995)	Vandenberg et al., 2004
Belgium	2008 to 2013	*A. butzleri, A. cryaerophilus, A. thereius*	Suspected gastroenteritis patients	Culture enrichment & isolation, PCR	0 to 99	1.3% (89/6,774)	Van de Abeele et al., 2014
Canada	2008	*A. butzleri*	Diarrheic stools (PCR) Diarrheic stools (culture) Non-diarrheic (PCR)	PCR, culture enrichment & isolation	0 to >65	57.0% (845/1,482) 0.8% (12/1,482) 45.5% (40/88)	Webb et al., 2016a
Chile	2010 to 2012	*A. butzleri*	Diarrheic stools (PCR) Diarrheic stools (culture) Non-diarrheic stools	PCR, culture enrichment, filtration onto blood agar	0 to >50	1.4% (2/140) 0.7% (1/140) 0.0% (0/116)	Collado et al., 2013

Table 1. Reported incidences of Arcobacter spp. detection in human stools

Area	Study Period	Species	Matrix	Method	Patient Age (y)	Percent Positive % (No. of Samples)	Reference
India	2009 to 2010	*A. butzleri, A. cryaerophilus, A. skirrowii*	Diarrheic stools (PCR) Diarrheic stools (culture)	PCR Selective enrichment, agar	0 to 97	4.0% (3/75) 2.7% (2/75)	Patyal et al., 2011
Italy	2005 to 2009	*A. butzleri, A. cryaerophilus*	Diabetic vs non-diabetic, all non-diarrheic stools	PCR	47 to 72	46.5% (46/99)	Fera et al., 2010a
Netherlands	2011	*A. butzleri*	Suspected gastroenteritis patients	qPCR[b]	0 to 97	0.4% (2/493)	de Boer et al., 2013
New Zealand	2007 to 2008	*A.butzleri, A. cryaerophilus*	Diarrheic stools	Selective enrichment, agar, PCR, PFGE[c]	2 to 78	0.9% (12/1380)	Mandisodza et al., 2012
Portugal	2012	*A. butzleri, A. cryaerophilus*	Diarrheic stools	qPCR (FRET[d])	0 to >65	1.7% (5/298)	Ferreira et al., 2014
S. Africa	1990 to 2000	*A. butzleri*	Diarrheic stools pediatric patients	Filtration onto blood agar, H2 enriched atmosphere	0 to 19	0.1% (16/19,535)	Lastovica and Roux, 2000

[a]PAGE: Polyacrylamide gel electrophoresis; [b](q)PCR: (quantitative) Polymerase chain reaction; [c]PFGE: Pulsed-field gel electrophoresis; [d]FRET: Förster resonance energy transfer.

Non-culture based methods used to screen for Arcobacter carriage in stool samples rely on the DNA extraction of fecal samples followed by PCR detection and/or quantification. In one study of ~500 random stool samples submitted for bacteriological testing, 0.4% tested positive for A. butzleri by PCR but subsequent recovery by culture was unsuccessful (de Boer et al., 2013). There are few studies for undeveloped regions, therefore rates of Arcobacter carriage are difficult to determine in these areas. One study performed in South Africa observed that ~13% of hospital patients were positive for Arcobacter carriage, using molecular detection methods, compared to ~3% in (healthy) school children (Samie et al., 2007). Further studies in under-developed regions will be necessary to determine if Arcobacter carriage rates are significantly different than those in developed countries studied to date.

Studies of traveller's diarrhea patients using PCR on fecal DNA extracts have also identified Arcobacter in 8% of diarrheal stools (Jiang et al., 2010). Hopefully, with the inclusion of Arcobacter in routine pathogen screening for traveller's diarrhea more accurate data will become available to determine its true impact. Arcobacter has also been cultured from the feces of healthy individuals involved with the handling, slaughter and transportation of animal products. Arcobacter was isolated from 1.4% of healthy donors, but required enrichment for isolation (Houf and Stephan, 2007). This was likely due to the observation that these individuals were shedding less than 100 CFU of arcobacters/g of feces. Only A. cryaerophilus was recovered in this study confirming that low-level Arcobacter carriage in humans may be asymptomatic, which likely warrants further study in determining true Arcobacter carriage rates across healthy individuals. A recent study performed in Chile found that A. butzleri was the 4th most common amongst the detected campylobacters with an incidence of 1.4% in patients with diarrhea, but was not detected in the healthy controls (Collado et al., 2013). In a recent study performed in Canada based on A. butzleri PCR-detection a similar incidence (56.7% vs. 45.5%) between diarrheic and non-diarrheic individuals was observed, but DNA concentrations in the diarrheic stools were significantly higher ($p<0.007$) (Webb et al., 2016a). The Arcobacter incidence in non-diarrheic stools in this study is much higher than that reported in other studies described above.

There are still knowledge gaps regarding the virulence potential of *Arcobacter* infections. However, work reported to date is suggestive of *Arcobacter* spp. having the potential to adhere to and invade several cell lines including human intestinal epithelial cells causing cytotoxicity (Collado and Figueras, 2011; Levican et al., 2013c; Ferreira et al., 2015; Karadas et al., 2016). Putative virulence genes have been identified in various *Arcobacter* species/strains in numerous studies (Douidah et al., 2012; Levican et al., 2013c; Ferreira et al., 2015; Girbau et al., 2015; Piva et al., 2017). Further work is required to better understand the relationship between these putative virulence markers and human clinical outcomes, though some tantalizing evidence suggests that *Arcobacter* has the potential to induce tight junction dysfunction (Bücker et al., 2009; Karadas et al., 2016), which may lead to diarrhea (Collado and Figueras, 2011). This is similar to the effect observed with enteroaggregative *E. coli* (EAEC) infection (Strauman et al., 2010).

1.3.2.2 Livestock - pigs/cattle/horses/sheep

Arcobacter has repeatedly been identified in the feces of healthy livestock animals. Cattle consistently appear to carry *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Wesley et al., 2000; van Driessche et al., 2005; Merga et al., 2011; Shah et al., 2013; Grove-White et al., 2014). Isolation rates vary between studies, but young cattle/calves were consistently shown to have higher *Arcobacter* carriage rates. Co-colonization with different *Arcobacter* species is also common. In these farming operations *Arcobacter* is also commonly detected in water samples and on the house floor of dairy operations. Having individual watering stations for dairy cows was shown to be protective against *Arcobacter* transmission, suggesting that water in dairy operations is a likely source of cross-infection (Wesley et al., 2000). Herd size is not likely related to infection rates, but source of feed may be. Additionally, housing of herds versus pasturing may lead to increased rates of *Arcobacter* carriage within herds (Grove-White et al., 2014), presumably due to the close proximity of the animals. Animals may carry multiple species, but detection of co-colonization may be difficult if performing culture enrichment. Direct plating from feces has been reported to have higher success at detecting co-colonization whilst lowering overall detection rates (Van Driessche et al., 2003). In the same study, post-enrichment rates of *Arcobacter* detection in livestock animals ranged from equine (15.4%) to porcine (43.9%), with presence also observed in ovines (16.1%) and bovines (39.2%).

Dairy farms have been shown to have a high probability of *Arcobacter* contamination. Due to the complexity of dairy operations and the focus on a liquid product (milk), there is a multitude of locations for potential *Arcobacter* contamination. In multiple studies *Arcobacter* has been identified in bulk milk tanks, milking apparatus, animal-watering stations, animal feed, barn floors, cattle feces (Wesley et al., 2000; Yesilmen et al., 2014; Giacometti et al., 2015a) and inline filters in milking apparatus (Serraino et al., 2013a). *Arcobacter* has also been identified in water buffalo milk (Giacometti et al., 2015b) and sheep dairy operations (Scarano et al., 2014). High rates of isolation of *Arcobacter* in dairy operations may be of significant concern to people due to the increasing popularity of artisanal dairy products (milk/cheese) that utilize raw milk. *Arcobacter* has been recovered from retail cheese (Scarano et al., 2014; Yesilmen et al., 2014) and raw milk (Revez et al., 2013; Yesilmen et al., 2014; Giacometti et al., 2015a), highlighting a direct linkage to human infection through consumption of contaminated foodstuffs.

Pigs are also a common source of *Arcobacter*, including several novel species. *A. lanthieri* (Whiteduck-Léveillée et al., 2015b) and *A. trophiarum* (De Smet et al., 2011) have been isolated from pig manure, *A. suis* from pig meat (Levican et al., 2013a), *A. cibarius* from piggery effluent (Chinivasagam et al., 2007) and *A. thereius* from the organs of spontaneous pig abortions (Houf et al., 2009). Several previously identified strains of *A. thereius* isolated from pork have recently been recognized as the novel species *A. porcinus* (Figueras et al., 2017). The zoonotic species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have also been found in the digestive tracts and cloacae of healthy pigs (Van Driessche et al., 2004; Ho et al., 2006; De Smet et al., 2011; De Smet et al., 2012). From research to date, pigs appear to be the livestock animal most consistently associated with *Arcobacter*.

1.3.2.3 Non-livestock animals

Arcobacter has been identified in a wide range of non-livestock mammals including alpaca, gazelle, rhinoceros and gorilla (Wesley and Schroeder-Tucker, 2011). *A. butzleri* has been isolated from a colony of rhesus macaques that displayed recurrent watery diarrhea (Higgins et al., 1999). Dogs have also been shown to carry human-associated species *A. butzleri* and *A. cryaerophilus* (Houf et al., 2008). This is potential concern due to the close proximity of companion animals and previous reports detailing an increased risk of Campylobacteriosis amongst dog owners (Mughini Gras et al., 2013). Reptiles have also recently been shown to carry *Campylobacteracea*, including *Arcobacter*. Lacertilia (lizards), Serpentes (snakes) and Testudines (chelonians) have all been shown to carry the human-associated species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Gilbert et al., 2014). These animals, like dogs, are often kept as companion animals and fecal-oral transmission is possible if proper hygiene is not followed after handling. There are likely many additional animals that carry *Arcobacter* that have not yet been described.

1.3.2.4 Poultry

Poultry produced for human consumption is likely to be a significant source of *Arcobacter*, much like *Campylobacter*. Several studies have shown rates of *Arcobacter* isolation from processed chicken meat/carcasses at rates of up to 100% (Atabay et al., 1998; Son et al., 2007; Rahimi, 2014). Rates of *Arcobacter* isolation are variable as there is currently no consistent isolation method adopted for culture/isolation. Based on current reports, the *Arcobacter* species commonly isolated

from chicken are *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, in decreasing frequency of detection. This frequency of detection is similar to the species detected from human stool samples (see above). For many years it was unclear as to the source of *Arcobacter* during poultry slaughter. There has been a debate as to whether or not *Arcobacter* is carried in the gut of chickens, as in several studies researchers were unable to culture/identify *Arcobacter* from fresh chicken ceca and suggested that process water may be the source of *Arcobacter* contamination on chicken carcasses (Van Driessche and Houf, 2007a). However, subsequent studies demonstrated that these microbes can inhabit the chicken intestine, but that the age of the sampled animals and the method used for recovery and identification influence the prevalence of *Arcobacter* (Ho et al., 2008). Other studies utilizing both PCR and culture confirmation have been able to identify *Arcobacter* in a large percentage of chicken intestines, though the rates of detection vary significantly between flocks (Lipman et al., 2008). *Arcobacter* has also been identified in poultry house litter at high levels, but was not ubiquitous across all houses (Dumas et al., 2011), consistent with other reports of sporadic detection in chicken feces.

Geese (Atabay et al., 2008), ducks and turkeys (Collado et al., 2009b) have also been shown to carry the three human-associated *Arcobacter* species (*A. butzleri*, A. *cryaerophilus*, *A. skirrowii*). Like chicken, the risk of infection is due to improper hygiene after handing of raw meat and/or consumption of undercooked meat. A foodborne outbreak in a wedding reception was associated with consumption of broasted chicken contaminated with *Arcobacter,* demonstrating direct linkage between poultry consumption and human GI disease (Lappi et al., 2013). As geese and ducks are waterfowl, they may represent a significant source of *Arcobacter* contamination in still bodies of fresh water.

1.3.2.5 Shellfish

At least six species of *Arcobacter* have been recovered from bivalves. *A. molluscorum* and *A. bivalviorum* have been recovered from mussels, clams, cockles and oysters (Figueras et al., 2012; Salas-Massó et al., 2016); *A. venerupis* from clams (Levican et al., 2012) and *A. ebronensis, A. ellisii*, *A. bivalviorum* and *A. mytili* have all been recovered from mussels (Collado et al., 2009a; Figueras et al., 2011a; Figueras et al., 2011b; Levican et al., 2012). Recently, *A. marinus*, *A. halophilus* and *A. mytili* have been recovered from oysters (Salas-Massó et al., 2016). A recent report determined that *Arcobacter* is present in high levels within the gut of laboratory-cultured sea urchins (Hakim et al., 2015), which could suggest a connection to bi-valves as mussels are a part of the diet of sea urchins and share the same ecosystem. It is unclear whether these species are residents of the gastrointestinal system of bivalves or accumulate within them due to their nature as filter feeders. As some of these shellfish are consumed raw/poorly cooked, potential transmission to humans is of concern especially if shellfish beds are impacted by sewage discharge or urban runoff. This phenomenon has been observed as high levels of *Arcobacter* spp. in marine waters have been directly correlated to fecal pollution levels entering the sea from a contaminated stream (Collado et al., 2008). As *Arcobacter* is relatively salt tolerant it has the potential to survive long enough to be captured by filter feeders, which could be later consumed by people leading to gastrointestinal illness. Human-associated *Arcobacter* spp. (*A. butzleri, A. cryaerophilus, A. skirrowii, A. thereius*) could potentially be transmitted back to people through this transmission cycle. Recently, *Escherichia coli* has been suggested as an index organism for predicting the incidence of *A. butzleri* contamination in bivalve mollusks because the latter species was detected in almost 50% of the shellfish samples that showed levels of *E. coli* >230 MPN/100 g, which is the standard above which shellfish require depuration before commercialization (Leoni et al., 2017).

1.3.2.6 Water

Water is a likely key component to *Arcobacter* transmission and in most studies looking at carriage rates in animals the associated water supplies commonly had *Arcobacter* present. This is particularly common in intensive farming operations where water is consumed by the animals or used to wash animal carcasses or vegetable produce (Hausdorf et al., 2013).

Arcobacter has been shown to possess the ability to form biofilms, which greatly impacts its ability to survive on abiotic surfaces (Houf et al., 2002; Ferreira et al., 2013). *Arcobacter* have been identified in water distribution pipe biofilms (Assanta et al., 2002) and on slaughter equipment in poultry abattoirs (Houf et al., 2002; Ferreira et al., 2013). This, coupled with the observation that *Arcobacter* can survive long periods in water (Van Driessche and Houf, 2008), or even replicate at slaughter and refrigeration temperatures 4 to 10 °C (Kjeldgaard et al., 2009) could place it in a very different level of risk compared to the other campylobacters.

Arcobacter in soil is most likely the result of fecal deposition from animals, watering with contaminated water, spreading of manure or release of sewage/septage from damaged or cross-connected systems and open defecation. Following rainfall this material may make its way into the aquifers or nearby waterways leading to potential human exposure. An overview of the global prevalence of *Arcobacter* in water has been presented by Hsu and Lee (2015). In addition, the association of *Arcobacter* with unicellular protists and plankton of various sizes has also been described (Maugeri et al., 2004; Hamann et al., 2016).

Arcobacter has been associated with at least four waterborne outbreaks. Two outbreaks were linked to contaminated well water with one of them being the first U.S. report of *A. butzleri* isolation from groundwater (Rice et al., 1999; Fong et al., 2007). A subsequent report described an outbreak of gastroenteritis that occurred after a drinking water network was been connected to a new building (Kopilovic et al., 2008) and another with a water distribution pipe breakage (Jalava et al., 2014). In three of these outbreaks multiple different pathogens were isolated from the tested water and/or the collected stool samples. This is consistent with the fact that the water was likely contaminated with sewage containing various pathogens

(Fong et al., 2007; Kopilovic et al., 2008; Jalava et al., 2014). Failures with the chlorination process used for disinfection of drinking water has been considered the cause of the presence *Arcobacter* in the water and the cause of at least one outbreak (Rice et al., 1999).

1.3.3 Incubation period

There are limited data available regarding *Arcobacter* infections making it difficult to make definitive conclusions regarding incubation periods. However, one report of a putative *Arcobacter* outbreak at a wedding reception suggested that symptoms may be observed as little as 6 hours and as long as three days post ingestion (Lappi et al., 2013). The mean incubation period for this outbreak was approximately 31 hours.

1.3.4 Period of communicability

As *Arcobacter* culture from stool is still an immature procedure there is limited data available on the levels shed from humans with *Arcobacter* infections. Asymptomatic *A. cryaerophilus* shedding has been reported to be <100 CFU/g of feces in seven individuals associated with animal slaughtering, meat handling and/or transportation (Houf and Stephan, 2007). A report from an Italian elementary school suggested the likelihood of person-to-person transmission of *A. butzleri* over a period of approximately three weeks with 10 cases in total (Vandamme et al., 1992b). Little else has been reported regarding person-to-person transmission amongst people, though it is likely analogous to *Campylobacter* in this regard. The infectious dose of *Arcobacter* spp. in humans is currently unknown, though is likely variable based upon species and/or strain.

In developing countries where sanitation and hygiene are poor and interaction with animals is frequent, person-to-person transfer between people is significant, particularly in children. *Campylobacter* carriage rates may be as high as 25% in children less than five years old, who may be asymptomatic (Coker et al., 2002). Diarrhea associated with *A. butzleri* seems more persistent and watery, but less acute and possibly more asymptomatic than associated effects from *C. jejuni* infections (Vandenberg et al., 2004). In fact recurrent episodes with abdominal pain seems to be the typical clinical presentation for *Arcobacter* (Figueras et al., 2014).

There are reports describing both vertical (mother to infant) and horizontal (animal to animal) transmission of *Arcobacter* between sows and piglets. Pregnant sows have been shown to carry *A. cryaerophilus* in their amniotic fluid, which is transmitted to their newborn piglets (Ho et al., 2006). However, sows were also shown to carry *A. skirrowii* or *A. butzleri* in their rectum and within three weeks the piglets had generally shed the *A. cryaerophilus* signature in favor of *A. skirrowii* and/or *A. butzleri* presumably acquired by the fecal-oral route due to proximity to the sows.

1.3.5 Population susceptibility

There are currently no reports describing susceptibility to *Arcobacter* infection in people. However, *Arcobacter* spp. together with other *Campylobacter* related organisms are considered important pathogens associated with diarrhea among HIV positive individuals from the Venda region, Limpopo, South Africa (Samie et al., 2007). Another HIV-positive study group had an *Arcobacter* incidence by culture of 2.67% (Patyal et al., 2011). However, repeated exposure to *Arcobacter* may contribute to immunity in healthy individuals. This may be possible considering that the phenomenon has been described for *Campylobacter* immunity, particularly in developing regions where sanitation and hygiene are poor and in developed regions amongst farm workers (Coker et al., 2002; Forbes et al., 2009). A report describing asymptomatic carriage of *A. cryaerophilus* in workers associated with meat handling supports this hypothesis (Houf and Stephan, 2007). Further studies are required to determine accurate asymptomatic carriage rates for *Arcobacter* in humans.

In Chile carriage rates of ~5% of *Campylobacter*-related organisms in non-diarrheic patients have been reported (Collado et al., 2013). However, *Arcobacter* was not detected in any of the controls, and was only found by molecular methods in two patients with diarrhea (1.4%) and in only one (0.7%) by culture. Conversely, in a Canadian study using molecular detection only, *Arcobacter* prevalence was very high, and there was little difference (57% vs. 46%) in *Arcobacter* detection rates between diarrheic and non-diarrheic patients (Webb et al., 2016a). The highest reported rate of *Arcobacter* detection to date is 9% in a group of Type 2 diabetic patients when using molecular methods (Fera et al., 2010b). In these same patients culture was only positive in 8%, reinforcing the idea that different *Arcobacter* detection methods may produce vastly different results, even in the same patient group. As such, care must be taken when comparing *Arcobacter* prevalence data between studies using different detection methods. Further studies, and more standardized methods will be required to better determine *Arcobacter* carriage rates in symptomatic vs. asymptomatic people.

1.4 Population and Individual Control Measures

1.4.1 Vaccines

To date there have been no reports regarding vaccine development targeting *Arcobacter*.

1.4.2 Hygiene measures

No information available, but same as for campylobacters recommended.

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods

Arcobacter was originally isolated from livestock abortions through the use of *Leptospira* culture methods and were described as aerotolerant *Campylobacter* (Neill et al., 1978). Later it was determined that due to their aerotolerance and lower optimal growth temperatures that

these isolates should form their own genus, *Arcobacter* (Vandamme, 1991). Since that time various methods have been developed to isolate *Arcobacter*. All are relatively similar to *Campylobacter* isolation methods, with the exception of temperature (Merga et al., 2011). Media bases such as Mueller-Hinton broth, Brucella broth and Bolton broth have been used. *Arcobacter*-specific broth (ASB) has also been marketed and is coupled with the CAT (Cefoperazone, Amphotericin B, Teicoplainin) selective supplement (Collado and Figueras, 2011). Antibiotics used in *Arcobacter* broths are often overlapping with those used in *Campylobacter* isolation. Freezing of fecal specimens is not recommended as it may produce a reduction of ~50% in the recovery of *Arcobacter* species (Merga et al., 2011) Sequencing of the *A. butzleri* genome revealed the presence of a large number of antibiotic resistance genes (Miller et al., 2007). In fact, *A. butzleri* RM4018 displayed resistance to 42 of 65 antibiotics tested, more than *Campylobacter jejuni*, *C. coli* or *C. lari*. It is therefore likely that temperature acts as the most important selective pressure in *Arcobacter* vs. *Campylobacter* culture. On this basis, recovery of *Arcobacter* from traditional *Campylobacter* media (Bolton and Preston broths) is not surprising and has been reported (Diergaardt et al., 2004; Merga et al., 2011; Figueras et al., 2014; Banting et al., 2016). *Arcobacter* is routinely cultured at 20 to 37°C under aerobic or microaerophilic conditions, with the most frequent temperature being 30°C, while *Campylobacter* spp. will not be effectively cultured at 30°C or below. Interestingly, an incubation temperature of 25 °C was used in a large Belgian study (Van den Abeele et al., 2014), which found an *Arcobacter* spp. incidence of 1.3% in cases with diarrhea. This incubation temperature was used, as it was the only incubator available with a temperature lower than 37°C (Van den Abeele personal communication). Thermotolerant *Campylobacter* are typically cultured at 42°C, but only a handful of *Arcobacter* spp. have the ability to grow at this temperature and this may even be strain dependent (Levican et al., 2013a). The majority of *Arcobacter* spp. will also grow in the presence of atmospheric oxygen, which will suppress *Campylobacter* growth.

Many of the reports describing *Arcobacter* detection (Table 2) are presence/absence only, through the use of culture enrichment or qPCR (Fera et al., 2010a; Collado et al., 2010; Ertas et al., 2010). A few quantitative studies have been described including detection of *Arcobacter* in the feces of healthy cattle (van Driessche et al., 2005), from piggery effluent (Chinivasagam et al., 2007) and from irrigation and wastewater (Banting et al., 2016; Fernandez-Cassi et al., 2016; Webb et al., 2016b). In piggery effluent *Arcobacter* levels ranged between 10^5 to 10^8 MPN/100mL (Chinivasagam et al., 2007). In irrigation water levels averaged ~33 MPN/100 mL, while in wastewater *A. butzleri* was detected at levels up to 10^5 MPN/100 mL (Banting et al., 2016; Fernandez-Cassi et al., 2016; Webb et al., 2016b). Using the enumeration method described by the Chinivasagam, others have found concentrations of $3.7x10^5$ MPN/100 mL of *Arcobacter* in a fecally contaminated freshwater stream (Collado et al., 2008). More recently using the same protocol, the *Arcobacter* concentration in secondary and tertiary (after a lagooning storage process) treated wastewater was $7.5x10^6$ MPN/100 mL and $4.6x10^2$ MPN/100 mL in outlet water, respectively (Fernandez-Cassi et al., 2016). The high concentrations of *Arcobacter* found in raw sewage or secondary treated wastewater enable the direct recovery of the bacteria (by passive filtration through 0.45 µm filters on blood agar) without an enrichment step, which will be a more objective approach to determine the dominant species (Levican et al., 2016). A quantitative miniaturized MPN-qPCR assay has been described for *Campylobacter* enumeration from surface and wastewater samples that also proved to be effective for recovery/enumeration of *A. butzleri* (Banting et al., 2016). Further optimization of this assay may lead to effective methods for quantitation of *Arcobacter* spp. from water matrices. A qPCR approach using PMA to inhibit amplification of DNA coming from dead bacteria has been developed (Salas-Massó et al. in preparation) which may allow more accurate quantitation of live *Arcobacter* by qPCR, as this bacterium, like others, is known to enter into a viable but non-culturable (VNBC) state when under stress (Fera et al., 2008). *A. butzleri* has been shown to be capable of resuscitation in rich media after up to 270 days storage in seawater at 4°C (Fera et al., 2008). As new *Arcobacter* species are identified/characterized, more optimized culture conditions are likely to be defined. However, growth requirements are unlikely to be standardized across the entire genus, as shown by specific growth requirements such as the salt requirements of *A. halophilus*, or the anerobiosis of *A. anaerophilus*.

Biochemical assays, PCR and/or DNA sequencing are typically performed to confirm strains as belonging to the genus *Arcobacter*. Typical biochemical responses for the genus are: positive catalase and urease activities, along with the ability to reduce nitrate and with a few exceptions the ability to hydrolyze indoxyl acetate. In addition, isolates are commonly tested for aerotolerance, resistance to NaCl, surfactants, tetrazolium chloride and various antibiotics. There are a variety of PCR assays described for the identification and speciation of *Arcobacter* targeting the 16S, 23S, *gyrA* and *hsp60* genes (Collado and Figueras, 2011; Levican and Figueras, 2013b). The resolving power of these assays is variable and none of the assays reported to date have shown the ability to correctly speciate all *Arcobacter* strains (Levican and Figueras, 2013b). In fact, the majority of studies use methods that purportedly target only three species (*A. butzleri, A. cryaerophilus* and *A. skirrowii*); however, using these methods other species have been also been identified (Levican and Figueras, 2013b). The 16S rRNA-RFLP method described by Figueras et al. (2008) and updated in 2012, is the only method other than DNA sequencing able to differentiate the majority of the species of the genus (Figueras et al., 2012). However, with the rapidly expanding nature of the *Arcobacter* genus, all methods will require further evaluation to determine their efficacy. Currently, DNA sequence analysis of selected genes (*rpoB*, *gyrB*, *cpn60* etc.) is the most accurate method for *Arcobacter* species assignment. This analysis can be done by BLAST comparison with sequences available in GenBank, but the construction of a phylogenetic tree that includes the new sequences along with those of the type strains of accepted species is a more reliable approach.

2.2 Data on Occurrence

2.2.1 Municipal sewage and human feces

There is an increasing body of literature detailing that *Arcobacter* is a consistent component of human sewage systems (Stampi et al., 1993, 1999; Moreno et al., 2003; González et al., 2007, González et al., 2010; Collado et al., 2008, 2010; McLellan et al., 2010; Fisher et al., 2014; Merga et al., 2014; Banting et al., 2016; Fernandez-Cassi et al., 2016; Webb et al., 2016b), likely able to replicate outside of a vertebrate host within the sewage infrastructure system and wastewater treatment plants (McLellan et al., 2010; Shanks et al., 2013, Fisher et al., 2014). This supports the observation of the levels of *Arcobacter* found in wastewater being much higher than expected based on human carriage rates. Human-associated *Arcobacter* species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have all be isolated from sewage samples by culture based methods (Stampi et al., 1993, 1999; Moreno et al., 2003; González et al., 2007, 2010; Collado et al., 2008; Levican et al., 2013a; Merga et al., 2014; Levican et al., 2016), and presumably could be present in human excreta in waterless sanitation or open defecation situations. As noted above, sewage has been the origin of some of the recently described new species in the genera. Utilization of metagenomic sequencing of sewage DNA extracts using pyrosequencing or next-generation sequencing technologies has revealed that *Arcobacter* is highly prevalent in sewage (McLellan et al., 2010; Van de Walle et al., 2012; Shanks et al., 2013; Cai et al., 2014; Fisher et al., 2014). *Arcobacter* has been identified in wastewater treatment plants globally and is reported to represent from 2% of total sewage partial 16S RNA gene sequences (Cai et al., 2014) up to 85% in some samples when the target region of the 16S gene was V4-V5 (Fisher et al., 2014). This proportion will be influenced by the region targeted within the 16S RNA gene (V1 to V9), which may explain the variable rates/concentrations observed in different metagenomic studies. Due to the nature of metagenomic sequencing using short fragments of the 16S RNA gene, speciation as operational taxonomic units (OTUs) is not always possible because of the lack of sequence diversity between closely related species. However, based on oligotyping sequence analysis of wastewater samples, it has been observed that two oligotypes that matched 100% to two different subgroups of the species *A. cryaerophilus* (subgroups 1B and 1A, respectively) were dominant, while the oligotype that corresponded with *A. butzleri* was only infrequently observed (Fisher et al., 2014). These results are in agreement with those reported when using culture by direct plating without enrichment (Figueras, unpublished results). Fisher et al. (2014) also reported a number of oligotypes that did not much with any of the existing species and could represent additional, un-described species in the genus *Arcobacter* from sewage.

The sewage ecosystem may also play a role in the amount and type of *Arcobacter* species found. Temperature seems to play a role in the type of *Arcobacter* identified in sewage, with some types/species only being identified when temperatures are low (<20°C) (Fisher et al., 2014). This may lead to seasonal changes in *Arcobacter* levels/composition in sewage depending upon location. Non-human sewage has also been shown to carry *Arcobacter* (Chinivasagam et al., 2007).

2.2.2 Sludge

Total *Arcobacter* was reported at levels of 10^5 MPN/g in primary sludge with a 2 $\log_{10}$ reduction when anaerobically digested (Stampi et al., 1999). An earlier report quantitating the single species *A. cryaerophilus* within sludge, showed a 1 $\log_{10}$ reduction (10^3 to 10^2 MPN/g) during anaerobic sludge digestion (Stampi et al., 1993). A subsequent report was able to isolate *Arcobacter* from 96% of sewage sludge samples, with *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* being the species recovered (Collado et al. 2008). Recently, *Arcobacter faecis* sp. nov. was identified in a domestic septic tank (Whiteduck-Léveillée et al., 2015a). Several reports have detected or quantified *Arcobacter* within the activated sludge of wastewater treatment plants (Stampi et al., 1999; Collado et al., 2008), with no apparent reduction during that secondary treatment.

2.2.3 Animal manures

Arcobacter has been isolated from pig and cattle manure from which the recently proposed new species *Arcobacter lanthieri* has been described (Whiteduck-Léveillée et al., 2015b). Effluent from piggery operations has been shown to carry high levels (up to 10^8 MPN/100 mL) (Chinivasagam et al., 2007). Piggery effluent is typically held in retention ponds, which may subsequently be applied to pastures nearby. Soil collected from these irrigated pastures also contained culturable *Arcobacter* at levels up to 10^4 MPN/g of soil. The two most prevalent *Arcobacter* species identified were known to be zoonotic (*A. butzleri* and *A. cryaerophilus*) indicating a possible risk associated with dispersal of lagoon waste from piggery operations (see section III below).

Chicken manure has also been identified as a source of *Arcobacter* spp. (He et al., 2013). Chicken litter taken from the floor of a chicken house that harbored 1 to 3 flocks has also been shown to contain *Arcobacter* using a metagenomics analysis (Dumas et al., 2011). Poultry litter is commonly recycled as a fertilizer or as feed for cattle, which may contribute to the spread of these zoonotic bacteria to cattle.

2.2.4 Surface waters

There have been numerous reports describing the detection of *Arcobacter* in surface waters (Table 2) such as rivers (Moreno et al., 2003; Collado et al., 2008; Collado et al., 2010), beaches (Collado et al., 2008; Lee et al., 2012), irrigation water (Banting et al., 2016) and WWTP effluent (González et al., 2007, 2010; Collado et al., 2008, 2010; McLellan et al., 2010; Cai et al., 2014; Fisher et al., 2014; Al-Jassim et al., 2015; Fernandez-Cassi et al., 2016; Gonzalez-Martinez et al., 2016; Webb et al., 2016b). *Arcobacter* detection in surface water correlates well with the amount of fecal indicator bacteria such as total and

fecal coliforms, intestinal enterococci, *E. coli* and *Clostridium perfringens,* suggesting a fecal origin (Collado et al., 2008, Collado et al.,2010; Lee et al., 2012; Fernandez-Cassi et al., 2016; Webb et al., 2016b).

Table 2. Summary of studies reporting the detection of *Arcobacter* spp. in different water matrices

Area	Study Period	Species	Matrix	Detection/ Speciation Method	Sample Volume (ml)	Percent Positive (# of Samples)	Concentration Average MPN[a] or gc[b]/100ml	Reference
Wastewater, sewage and sludge								
Australia (Queensland)	2002 to 2004	*A. butzleri* *A. cryaerophilus* *A. cibarius*	Raw sewage (piggery effluent)	MPN culture enrichment, plating, mPCR, 16S rDNA sequences	1000	100% (14/14)	2.6E+07 MPN	Chinivasagam et al., 2007
Canada (Alberta)	2014 to 2015	*A. butzleri*	Raw sewage	Centrifugation, MPN culture enrichment, qPCR[c]	100	100% (2/2)[d]	1E+05 MPN	Banting et al., 2016
South Africa	NR[e]	*A. butzleri*	Raw sewage	Filtration, culture enrichment, plating, 16S rDNA[f] sequences	100	25% (1/4)	Presence/ absence	Diergaardt et al., 2004
Spain	2000	*Arcobacter* spp.	Raw sewage Secondary treated Activated sludge	Culture enrichment, PCR[h] and FISH[i]	300	100% (10/10) 80% (8/10) 100% (10/10)	Presence/ absence	Moreno et al., 2003
Spain	2006	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Raw sewage Activated sludge	Culture enrichment, plating, mPCR[j] & 16S rDNA-RFLP[k]	200 1	100% (19/19) 96% (26/27)	Presence/ absence	Collado et al., 2008
Spain	2007	*A. butzleri* *A. cryaerophilus*	Activated sludge	Culture[k] enrichment mPCR PCR	25	67% (10/15) 67% (10/15) 100% (15/15)	Presence/ absence	Gonzalez et al., 2007
Spain	2008	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Raw sewage	Filtration, culture enrichment, plating, mPCR & 16S rDNA-RFLP	200	100% (15/15)	Presence/ absence	Collado et al., 2010
Spain	2009 to 2011	*A. butzleri* *A. cryaerophilus* *A. nitrofigilis* *A. defluvii* *A. cloacae*	Raw sewage	Direct plating, enrichment, filtration, culture, mPCR/ mPCR & 16S rDNA-RFLP	200	95% (20/21)	Presence/ absence	Levican et al., 2016
Spain	2012 to 2013	*Arcobacter* spp.	Secondary treated WW Tertiary treated WW (lagooning)	Filtration, MPN culture enrichment	100	100% (12/12) 41.6% (5/12)	2.1E+07 MPN 3.5E+05 MPN	Fernandez-Cassi et al., 2016
Turkey	NR	*A. butzleri* *Arcobacter* spp.	Raw sewage	Filtration, culture enrichment, PCR, 16S sequencing	200	38% (25/66)	Presence/ absence	Akincioglu, 2011
UK	2011	*A. butzleri* *A. cryaerophilus*	Raw sewage	Culture enrichment, PCR, mPCR	NR	100% (9/9)	Presence/ absence	Merga et al., 2014

Area	Study Period	Species	Matrix	Detection/ Speciation Method	Sample Volume (ml)	Percent Positive (# of Samples)	Concentration Average MPN[a] or gc[b]/100ml	Reference
Other matrices								
Canada (Alberta)	2014 to 2015	*A. butzleri* *A. cryaerophilus*	Irrigation water	Centrifugation, MPN culture enrichment, qPCR	300	79% (63/80)	33 MPN	Banting et al., 2016
Italy	2007 to 2008	*A. butzleri* *A. cryaerophilus*	Estuarine water	Filtration, culture enrichment, PCR	2500	100% (4/4)	Presence/ absence	Fera et al., 2010b
Japan Thailand	2000 to 2002	*A. butzleri* *A. skirrowii*	River water Canal water	Selective culture/ PCR, rpoB-rpoC sequences		24% (4/17) 100% (7/7)	Presence/ absence	Morita et al., 2004
S. Africa	NR	*A. butzleri*	Tap water Ground water Surface water	Filtration, culture enrichment, plating, 16S rDNA sequences	100	0% (0/5) 0% (0/4) 64% (7/11)	Presence/ absence	Diergaardt et al., 2004
Spain	2000	*Arcobacter* spp.	River water	Culture enrichment, PCR and FISH	300	90% (9/10)	Presence/ absence	Moreno et al., 2003
Spain	2006	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	River Lake Seawater	Culture enrichment, plating, mPCR & 16S rDNA-RFLP	200	59% (17/29) 28% (8/29) 43% (43/101)	Presence/ absence	Collado et al., 2008
Spain	2008	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Clean river site Fecally polluted river Treated drinking water	Filtration, culture enrichment, plating, mPCR & 16S rDNA-RFLP	200	40% (2/5) 100% (30/30) 10% (1/10)	Presence/ absence	Collado et al., 2010
Turkey	2007 to 2008	*A. butleri* *A. skirrowii*	Treated drinking water Spring water	Culture enrichment, plating mPCR	20	3% (3/100) 4% (1/25)	Presence/ absence	Ertas et al., 2010
Turkey	NR	*A. butzleri.* *Arcobacter* spp.	River Drinking water	Filtration, culture enrichment, PCR, 16S sequencing	200	44% (11/25) 25% (6/24)	Presence/ absence	Akincioglu, 2011
USA (Ohio)	2004	*Arcobacter* spp.	Groundwater	Filtration, culture enrichment, plating, PCR-RFLP	4000	44% (7/16)	Presence/ absence	Fong et al., 2007
USA (Ohio)	2010	*Arcobacter* spp.	Beachwater (fresh)	Filtration, qPCR	200	75% (97/129)	1E+03 to 1E+04 gc	Lee et al., 2012

[a]MPN: Most probable number; [b]gc: Gene copies; [c]qPCR: Quantitative polymerase chain reaction; [d]A. butzleri only tested; [e]NR: Not reported; frDNA: Ribosomal RNA gene; [g]WW: wastewater; [h]PCR: Polymerase chain reaction; [i]FISH: Fluorescent in situ hybridization; [j]mPCR: Multiplex PCR (Houf et al., 2000); [k]rDNA-RFLP: Ribosomal RNA gene- restriction fragment length polymorphism (Figueras et al., 2012).

2.2.5 Groundwaters

Groundwater contamination with *Arcobacter* has been reported on several occasions, being associated with two gastroenteritis outbreaks: one from a child summer camp (Idaho, USA) and the other from South Bass Island, Ohio, USA (Rice et al., 1999; Fong et al., 2007). In the latter case, an exceptionally high amount of rainfall over a period of three months resulted in massive groundwater contamination, likely from local sewage and septic systems in the area. Some of the contaminated wells were auxiliary connected with the distribution system and *Arcobacter* was isolated from 7/16 of these wells via a *Campylobacter* culture isolation method and PCR identification. Each of these wells was also positive for, viruses, *E. coli* and total coliforms supporting the hypothesis that the source of the bacteriological contamination was sewage. *Arcobacter* was recovered from the wells that that had higher levels of fecal contamination.

In the summer camp outbreak in Idaho, the chlorination system failed, which was the single treatment barrier for the well water at the camp (Rice et al., 1999). In the outbreak >80% of the individuals staying at the camp became ill with gastroenteritis. The single well on site tested positive for both total and fecal coliforms and *A. butzleri* was isolated from the well. Two *A. butzleri* isolates from the well showed the ability to survive for at least 16 days at 5°C with less than 0.5 $\log_{10}$ loss of viability, but cells were sensitive to chlorination (Rice et al., 1999). In both outbreaks, it was suspected that contamination of the wells may have been present for some time prior to the detection and recognition of the outbreaks.

2.2.6 Drinking waters

Similar to groundwater, drinking water can be a source of *Arcobacter* as several reports detail the isolation of *Arcobacter* spp. from treated water (Jacob et al., 1993; Jacob et al.,1998; Ertas et al., 2010). In Turkey 3% of drinking water samples (chlorine treated) tested contained *A. butzleri* with 2% of the samples having culturable *A. butzleri* and *A. skirrowii* (Ertas et al., 2010). Another report of *Arcobacter* in drinking water described a waterborne outbreak in rural Finland in which the main drinking water pipe was broken during road construction and contaminated groundwater entered into the water line and made its way to a water storage reservoir, which was not disinfected (Jalava et al., 2014). Subsequent to the event *Arcobacter* was detected by PCR at two distribution system locations (one of which was a biofilm) and also by 16S rRNA gene sequencing of a water concentrate from the storage reservoir. However, no *Arcobacter* was recovered by culture-based methods.

A waterborne outbreak similar to the one described in Finland (Jalava et al., 2014) was reported in Slovenia after the drinking water system in a new building was connected. A. cryaerophilus was isolated from the feces of one (3.2%) of the 43 reported cases with acute gastroenteritis (Kopilović et al., 2008). In the latter outbreak as in the one of South Bass Island the gastroenteritis cases had multiple etiologies, due to the different pathogens present in the sewage assumed to have contaminated the drinking water, combined by different susceptibility of the exposed population on the basis of the immunity etc.

Arcobacter has been shown to possess the ability to form biofilms (Fernandez et al., 2008; Kjeldgaard et al., 2009; Ferreira et al., 2013) and has been identified in biofilms in drinking water distribution pipes (Assanta et al., 2002), which has the potential to contribute to gastrointestinal illness in people who consume such water.

2.2.7 Seawaters

Arcobacter has been isolated from seawater and marine shellfish (see 1.3.2.5 and references therein), including *A. butzleri* that may be associated with plankton (Fera et al., 2004; Maugeri et al., 2004). Other reports have identified all three human infectious species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* from seawater (Collado et al., 2008). The presence of these species of *Arcobacter* was correlated to fecal pollution entering the sea from contaminated freshwater streams as the presence of *Arcobacter* was directly correlated with the concentration of fecal indicator bacteria (Collado et al., 2008). Marine shellfish have also been shown to carry unique *Arcobacter* species (see section 1.3.2.5), but it is unclear if these arcobacters are commensal to the bivalves or accumulate in these filter feeders. Additional species like *A. marinus* (Kim et al., 2010), *A. pacificus* (Zhang et al., 2015), *A. ebronensis* and *A. aquimarinus* (Levican et al., 2015) have also been isolated from marine environments. Metagenomic analysis of the digestive tract and excreted pellets of sea urchins (*Lytechinus variegatus*) revealed the presence of *Arcobacter,* with some oligotypes not matching any known species (Hakim et al., 2015). As such, there will likely be further novel species identified from the marine environment in the near future.

Pyrosequencing of 16S rDNA from seawater DNA extracts in Korea revealed the presence of large amounts of *Arcobacter*, but this was observed in the summer season only (Suh et al., 2015). Using a qPCR approach, abundance of *Arcobacter* in Lake Erie beach water was negatively correlated with water temperature in agreement with previous research reporting that they survive better at lower water temperatures (Lee et al., 2012; Salas-Massó et al., 2016). *Campylobacter* has been observed to have a seasonal occurrence in environmental surface waters in summer/fall that were identified as being from a bovine or wild bird host (Strachan et al., 2013). As *Arcobacter* has a similar host distribution as *Campylobacter* it is possible that *Arcobacter* follows a similar temporal distribution. A tendency for certain species to show a seasonality pattern has been observed (Levican et al., 2014; Salas-Massó et al., 2016), as well as a peak in detection rates of arcobacters in diarrheic stools between June and October in Alberta, Canada (Webb et al., 2016a). Further surveillance data is required to determine the nature of such seasonality in *Arcobacter* recovery.

2.2.8 Soils

Arcobacter has been detected in soils impacted by wastewater. As noted above, in piggery operations wastewater is often lagooned and then spread on nearby fields to act as fertilizer. *Arcobacter* has been detected at levels up to 10^4 MPN/g in soils freshly irrigated with piggery lagoon effluent (Chinivasagam et al., 2007).

2.2.9 Irrigation water and on crops

A. butzleri and *A. cryaerophilus* have been detected in irrigation water samples in Alberta, Canada (Banting et al., 2016). *A. butzleri* averaged ~33 MPN/100 mL of irrigation water in between June and September, with a range from 0 to >800 MPN/100 mL.

Various species of *Arcobacter* have been detected in a spinach processing plant at multiple locations (Hausdorf et al., 2013). *Arcobacter* was detected in the process water and wash basins and the known potential pathogenic species *A. butzleri* was cultured from washed and blanched spinach, showing the potential for leafy green vegetables to be implicated in gastrointestinal disease. *Arcobacter* m-PCR detection after enrichment culture revealed that vegetables (tomatoes, parsley and lettuces) irrigated with reclaimed secondary treated wastewater disinfected with chlorine and UV were positive for *Arcobacter* in 14.3% of the samples from Spain, but the bacteria was not detected by culture (Figueras, personal communication).

2.2.10 Fish and shellfish

The presence of *Arcobacter* spp. in fish has only been reported sporadically. To date the limited reports have involved rainbow trout (*Oncorhynchus mykiss*), finfish and sea fish from retail markets from India (Collado and Figueras, 2011; Patyal et al., 2011; Ammar and Al Habaty, 2015; Laishram et al., 2016). However, as noted previously, shellfish are considered an important reservoir of arcobacters (see 1.3.2.5 and references therein). In recent years there have been several new *Arcobacter* species isolated from bivalve shellfish with ~9 new species pending description from this study (Salas-Massó et al., 2016). There are likely additional, undescribed, species in fish, shellfish and other marine organisms. Sewage associated arcobacters (*A. butzleri, A. cryaerophilus, A. skirrowii*) have also been isolated from shellfish (Levican et al., 2014; Salas-Massó et al., 2016), likely a result of fecal contamination into the marine environment (Collado et al., 2008) and capture by the filter feeding nature of bivalves. It has been demonstrated that shellfish (mainly oyster and mussels) can become contaminated with *Arcobacter* after a 4-day immersion in sewage contaminated channel water (Salas-Massó et al., 2016).

2.2.11 Air

No information was identified

2.3 Persistence

As *Arcobacter* displays aerotolerance, it is likely to have increased persistence/survivability in the environment as compared to more fastidious bacteria like *Campylobacter* spp. Additionally, arcobacters have been identified in irrigation water at a frequency and concentration much higher than *Campylobacter* (Banting et al., 2016), suggesting greater environmental survivability and/or growth. The fact that *Arcobacter* is found at such high levels in wastewater as compared to its prevalence in humans (Table 1) suggests that it has the ability to replicate outside of a vertebrate host within the sewer system or wastewater treatment plant (McLellan et al., 2010; Shanks et al., 2013, Fisher et al., 2014). It has been demonstrated that *Arcobacter* can survive full wastewater treatment, including UVB radiation (Webb et al., 2016b).

Recovery of *Arcobacter* from marine environments (Collado et al., 2008; Collado et al., 2009a; Levican et al., 2014; Salas-Massó et al., 2016) also supports the idea that its environmental persistence is greater than that of *Campylobacter* spp. Type strains of *A. butzleri* could not be grown in seawater nor bio-accumulated in mussels, under the tested conditions used by Ottaviani et al. (2013). However, the capacity of this species and others to bio-accumulate in shellfish after exposure to heavily contaminated water has been demonstrated in other studies (mentioned above in sections 1.3.2.5, 2.2.7 and 2.2.10).

The use of reclaimed irrigation water for ready to eat vegetables may increase the potential for human infection, especially considering that *Arcobacter* has been identified on packaged vegetables (Gonzalez and Ferrus, 2011; Fernandez-Cassi et al., 2016). Experimentally, *Arcobacter* has been shown to be extremely persistent in both water and food matrices. The survivability of *A. butzleri* in fresh water (river), combined with organic material, has been experimentally demonstrated to be greater than 200 days at 4°C (Table 3; Van Driessche and Houf, 2008). *Arcobacter* isolates from a groundwater source (well) linked to a GI outbreak showed little to no loss of viability over 16 days at 4°C (Rice et al., 1999). Similarly, in a seawater matrix, *A. butzleri* can persist for at least three weeks at 4°C (Table 3; Fera et al., 2008). In food matrices, *Arcobacter* survivability is equally high. *A. butzleri* can survive in chicken meat juice stored in a refrigerator (4°C) for at least 77 days with little to no loss of viability (Table 4; Kjeldgaard et al., 2009),

while survival in unpasteurized milk was shown to be 22% after 10 days at 4°C (Table 3; Serraino et al., 2013b). Additionally, A. butzleri and A. cryaerophilus showed the ability to survive after ultrahigh pasteurization process (Ferreira et al., 2015). As milk is typically consumed raw, this presents a real risk of human infection (Collado and Figueras, 2011; Serraino et al., 2013a; Ferreira et al., 2015).

Table 3. Loss of culturability for *Arcobacter* spp. in different matrices

Area	Microorganism	Matrix	Temp (°C)	Time to Loss of Culturability (Day)[a]	Reference
Belgium	*A. butzleri* (4 strains)	H_2O	4	169	Van Driessche and Houf, 2008
			20	123	
		H_2O + organics	4	227	
			20	149	
	A. cryaerophilus (4 strains)	H_2O	4	79	
			20	60	
		H_2O + organics	4	138	
			20	84	
	A. skirrowii (4 strains)	H_2O	4	72	
			20	32	
		H_2O + organics	4	121	
			20	39	
Canada	*A. butzleri*	Cattle manure	25	7	Inglis et al., 2010
	A. butzieri ATCC 49616	Filtered seawater	4	20	Fera et al., 2008
			20	14	
Italy	*A. butzleri* DSM 8739	Unpasteurized milk	5	~T80: 10 (22% viable)	Serraino et al., 2013b
			20	T90: 10 (10% viable)	

[a]As determined by colony forming units

Table 4. Persistence of *Arcobacter* spp. in different matrices

Area	Microorganism	Initial # (log_{10} CFU[a]/mL)	Post Storage # (log_{10} CFU/mL)	Days of Storage	Inactivation Rate (log_{10} CFU/day)	Temp (%C)	Matrix	Reference
Denmark	*A. butzleri* ATCC 49616	~9.2	~8.7	77	0.01	4	Chicken meat juice	Kjeldgaard et al., 2009
		~3.3	~8.5	20	-	10		
		~3.3	~8.5	10	-	15		
Italy	*A. butzleri* ATCC 49616	~7.5	~7.3	4	0.05	4	Filtered seawater	Fera et al., 2008
			~5.1	7	0.34	4		
			~3.8	12	0.31	4		
			~2.4	14	0.36	4		
			-1.0	20	0.33	4		
			~4.4	4	0.78	20		
			~4.0	7	0.5	20		
			~3.0	12	0.38	20		
			-1.0	14	0.46	20		
Spain	*A. butzleri* ATCC 12481	~7.5	~1.1	16	0.38	12	Drinking Water[c]	Moreno et al., 2004
		~7.5	~1.9	5 min contact time	NA[d]	12	CDW[e]	
USA	*A. butzleri* (2 isolates)	4.44 ± 0.02	4.04 ± 0.06	16	0.03	5	Well water	Rice et al., 1999
		6.06 ± 0.08	5.64 ± 0.06	16	0.03	5		

[a]CFU: Colony forming units; [b]ND: Not determined; [c]Drinking water no chlorine; [d]NA: Not applicable, log_{10} inactivation was 5.6; [e]CDW: chlorinated drinking water.

Arcobacter is, however, generally considered to be quite susceptible to traditional wastewater treatment (see Section 3.0; Table 5), though exceptions have been noted (Cai et al., 2014; Webb et al., 2016b). In addition, chlorine is highly effective at inactivation of *Arcobacter*. As little as 5 minutes in chlorinated drinking water can reduce culturable *A. butzleri* levels by >5 log_{10} (Table 4; Moreno et al., 2004). Traditional drinking water treatment typically involves chlorination, and removal of *Arcobacter* by traditional drinking water treatment/disinfection has been shown to be highly effective (as assayed by culture) (Collado et al., 2010). Despite treatment efficacy for removal of *Arcobacter*, recontamination may occur and result in outbreaks as noted above (Ertas et al., 2010; Collado and Figueras, 2011; Jalava et al., 2014).

3.0 Reductions by Sanitation Management

3.1 Excreta and Wastewater Treatment

3.1.1 Onsite sanitation

Soil treatment units (STU) receiving effluent from private septic tanks have been reported to be highly effective at removal of culturable *Arcobacter* (Tomaras et al., 2009).

3.1.1.1 Dry sanitation with inactivation by storage

No information identified, but note that arcobacters may grow if moisture and other conditions are favorable, as seen in wastewater treatment systems.

3.1.1.2 Pit latrines, vault toilets, dry toilets

No information identified, but note that arcobacters may grow if moisture and other conditions are favorable, as seen in wastewater treatment systems.

3.1.1.3 Composting

There are limited reports regarding the removal of *Arcobacter* by composting. Available data show that *Arcobacter* in chicken manure detected by non-quantitative PCR disappeared after 30 days of composting with temperatures >50°C (He et al., 2013). In a study involving bovine manure composting, *Arcobacter* was recovered by culture up to 7 days after formation of compost piles, after which point it could no longer be recovered (Inglis et al., 2010). Further studies are required using additional detection methods to determine more accurate removal kinetics of *Arcobacter* during composting.

3.1.1.4 Other residuals (solids) management: with the intention of reuse as fertilizer for agriculture/food, etc.

No information available, although *Arcobacter* has been detected at levels up to 10^4 MPN/g in soils freshly irrigated with piggery lagoon effluent water (Chinivasagam et al., 2007).

3.1.2 Waste stabilization ponds

The average reduction of *Arcobacter* after secondary treatment via lagooning has been reported to be ~99.99% (Fernandez-Cassi et al., 2016; Table 5).

Table 5. Treatment reductions of *Arcobacter* spp. in different sanitation processes

Area	Species	Matrix	Treatment	Quantitation Method	Volume Analyzed (ml)	Influent Log10 Concentration MPN[a] or gc[b]/100 ml	Effluent Log10 Concentration MPN or gc/100 ml	Log10 Reduction	Reference
China	*Arcobacter* spp.	Wastewater	Membrane bioreactor	23S qPCR[c]	1000	11.0 gc	8.16 gc	2.84	Yang et al., 2015
Italy	*A. cryaerophilus*	Wastewater	Activated sludge (MAS[d])	MPN culture	100	3.75 MPN	0.60 MPN	1.99	Stampi et al., 1993
			Post chlorination		20 (influent)			2.0	
Saudia Arabia	*Arcobacter* spp.	Wastewater	Activated sludge (MAS)	16S metagenomics	200 (2° effluent)	4.9 ± 4.9% gc	0.55 ± 0.2%	4.35	Al-Jassim et al., 2015
			Post chlorination		900		0.18 ± 0.18% gc	4.72	
Spain (Catalonia)	*Arcobacter* spp.	Secondary treated wastewater	Lagooning September sampling	MPN culture	0.5	8.04 MPN	ND[e]	8.04	Fernandez-Cassi et al., 2016
			April sampling			5.09 MPN	5.11 MPN	0	
USA (Ohio)	*Arcobacter* spp.	Primary treated wastewater	Peat biofilter	23S qPCR	2000	10.58 gc	7.52 gc	3.06	Park et al., 2016
			Post chlorination				7.87 gc	No reduction	

[a]MPN: Most probable number; [b]gc: Gene copies; [c]qPCR: Quantitative polymerase chain reaction; [d]MAS: Mixed activated sludge; [e]ND: Non detect for the month of September.

3.1.2.1 Aerated lagoons

No information available, but potential for some arcobacters to regrow (see Section 4.2).

3.1.3 Wetlands

Wetlands have been shown to contribute to the reduction of fecal indicator bacteria load within a near shore and freshwater swimming beach (Rea et al., 2015). Considering that the loads of fecal indicator bacteria predict the presence of arcobacters (Collado et al., 2008, 2010), it could be expected that *Arcobacter* may also reduce by wetlands, but given arcobacters ability to grow within sewage treatment systems, their removal or growth has yet to be reported.

3.1.4 Wastewater treatment and resource recovery facilities

3.1.4.1 Primary /preliminary treatment

There is limited data available regarding primary treatment of *Arcobacter* spp.. In a recent study, very little difference in the concentration of *Arcobacter* between the influent water and the primary sedimentation tank was observed (Levican et al., 2016). Despite this, it is generally has been assumed that treatment efficacy of *Arcobacter* will be similar to *Campylobacter* (see *Campylobacter* chapter). However, due to the evidence that indicates that *Arcobacter* may replicate in wastewater infrastructure (Fisher et al., 2014; Levican et al., 2016), this assumption may not be warranted.

3.1.4.2 Secondary treatment

Next generation sequencing (NGS) has allowed the collection of large amounts of sequence data from sewage treatment works. This has allowed the determination of the bacterial community diversity at each treatment step

(McLellan et al., 2010; Cai et al., 2014; Gonzalez-Martinez et al., 2016). As described by McLellan et al. (2010), sewage communities form a unique population structure represented by a combination of inputs (human fecal microbes and enrichment of specific microbes, largely from the environment). These, and other studies, have allowed the identification of microbial community signatures associated with sewage infrastructure. Within these bacterial community structures, several signature taxa have been identified (*Arcobacter* is considered a sewer-associated signature) that are informative about sources of fecal pollution (Newton et al., 2013).

The efficacy of treatment works can also be monitored using NGS approaches, by observing the removal of certain bacterial genera. For example, the Shatin WWTP in Hong Kong was shown to be highly effective at removing certain genera (*Streptococcus, Enterococcus, Blautia*), while others, including *Arcobacter*, were less susceptible to the biological treatment (Cai et al., 2014). In this study, *Arcobacter* was found to be the 4th most prevalent genus in treated effluent, though NGS technology does not offer insight into the viability of these bacteria. In a recent study, *Arcobacter* was reported as one of the most prevalent genera in the influent samples to the WWTPs studied (Gonzalez-Martinez et al., 2016). *Arcobacter* was the most abundant genus in the bioreactor (28%) and together with *Bacteroides* (25%) represented more than 50% of the total bacterial population. The potential ecological role attributed to *Arcobacter* in the bioreactor was BOD removal and de-nitrification (Gonzalez-Martinez et al., 2016).

Given the potential for arcobacters to grow during sewage treatment, it was interesting to see that a recent study of secondary treatment WWTP in Saudi Arabia showed (by NGS) effective removal of certain genera such as *Pseudomonas* (4 $\log_{10}$ reduction) and *Arcobacter* (2 $\log_{10}$ reduction) from influent to effluent water (Al-Jassim et al., 2015). A further 1 $\log_{10}$ removal of *Arcobacter* was achieved by chlorination.

Non-quantitative studies using culture enrichment and PCR have been able to isolate different *Arcobacter* spp. from secondary-treated wastewater effluent (Moreno et al., 2003; González et al., 2007, 2010; Collado et al., 2010; Levican et al., 2016). Average density of *Arcobacter* in a secondary treated water effluent entering a tertiary lagoon was 7.51x10^6 MPN/100 mL (Fernandez-Cassi et al., 2016) but higher values have also been reported (Levican et al., 2016). Despite the fact that significant numbers of viable *Arcobacter* may be released in secondary treated wastewater, drinking water treatment has been shown to be effective at removal of *Arcobacter* (Collado et al., 2010). For example, secondary treated wastewater containing live *Arcobacter* supplied to the intake of a drinking water treatment plant (DWTP), resulted in non-detects following flocculation, filtration, ozonation and chlorination treatment (Collado et al., 2010).

3.1.4.2.1 Trickling filters

No information identified, but potential for some arcobacters to regrow (as just described above).

3.1.4.2.2 Activated sludge

Activated sludge treatment is effective at removal of *Campylobacter*, but *Arcobacter* only displayed a 2 $\log_{10}$ removal following clarification, while actually increasing by 2 $\log_{10}$ during the activation stage (Stampi et al., 1999). An earlier report looking specifically at *A. cryaerophilus* only showed a 1 $\log_{10}$ removal of culturable cells (Stampi et al., 1993). As there is a variety of *Arcobacter* spp. in sewage, this data may not be representative activated sludge treatment efficacy on different arcobacters.

3.1.4.2.3 Oxidation ditch

No information available, but potential for some arcobacters to regrow (see Section 4.2).

3.1.4.2.4 Membrane bioreactors

A recent report describing the irrigation of crops (pakchoi) with treated wastewater from a membrane bioreactor WWTP showed a high concentration of *Arcobacter* spp. in the effluent and in the soil associated with the roots (rhizosphere) of these plants (Yang et al., 2015). Utilizing qPCR as a quantification method the membrane bioreactor produced a ~3 $\log_{10}$ removal of *Arcobacter* spp. during treatment (from 10^{10} 23S rRNA gene copies/mL to 10^7 copies/100 mL) (Table 5). At least 10^8 23S rRNA copies of *Arcobacter* spp./g of soil were detected in the root system of crops irrigated with membrane bioreactor treated wastewater (Yang et al., 2015), demonstrating potential human health risk associated with the consumption of these vegetables in a raw state.

3.1.4.2.5 Anaerobic/ anoxic digestion and biogas systems

No information available

3.1.5 Biosolids/sewage sludge treatment

No information available. See *Campylobacter* chapter.

3.1.6 Tertiary treatment

3.1.6.1 Lagooning

Lagooning is a natural (biological) treatment process of purifying wastewater by storing it in open air artificially made lagoons or stabilization ponds. The method attempts to be a more sustainable alternative to the more expensive and energy demanding tertiary treatment options such as tertiary clarifiers and disinfection using chlorination and ultraviolet light treatment. However, limited data has been identified on the efficacy of lagooning for removal of *Arcobacter*. Nonetheless, a recent paper demonstrated that from the high *Arcobacter* concentrations at the inlet (7.51x10^6 MPN/100 mL) there was a very significant (p value 6.21x10^{-4}) reduction of 4 $\log_{10}$ by lagooning. In fact, in more than 50% of the samples in which *Arcobacter* was not detected, viruses were also not detected, suggesting very effective general treatment (Fernandez-Cassi et al., 2016). However, non-detection of *Arcobacter* occurred predominantly during the colder months of the year (September to January) and the pattern of *Arcobacter* reduction followed that of traditional fecal indicators (Fernandez-Cassi et al., 2016). Therefore, lagooning may

represent an alternative, less expensive, treatment method, however the parameters that correlate with the reduction efficacy will need be thoroughly characterized in different geographic and climactic regions.

3.1.6.2 Coagulation
No information available

3.1.6.3 Filtration

3.1.6.3.1 Membranes/biofilters
A recent description of an on-site treatment system for a single home has shown that peat filtration is effective at *Arcobacter* removal (Park et al., 2016). The treatment system employed a septic tank followed by peat biofiltration and then chlorination. The chlorination tank utilized pumps to mix the effluent during the chlorination process to ensure effective chlorine distribution. Quantitation of *Arcobacter* spp. by qPCR determined that peat filtration produced a 3.1 $\log_{10}$ reduction, while chlorination did not provide any reduction (noting that PCR does not assess viability) (Table 5).

3.1.6.3.2 Mono, dual and tri media
No information available

3.1.6.4 Land treatment
No information available, yet note high concentrations in soils irrigated with piggery lagoon effluent (Chinivasagam et al., 2007).

3.1.6.5 Other processes
No information available

3.2 Disinfection as a Tertiary (or Post Primary) Treatment

3.2.1 Chlorine, combined etc.

Arcobacter has been shown to be susceptible to chlorination with at least 5 $\log_{10}$ reduction in culturability after a 5 minute exposure to chlorinated water (Rice et al., 1999; Moreno et al., 2004). At least one report described complete removal (to non-detection by the method used) of *Arcobacter* during drinking water treatment of water contaminated with *Arcobacter* (Collado et al., 2010). However, it would be worth examining larger volumes of the original water sample (concentrates of 1 to 1000 L of water) to increase the confidence of these observations.

3.2.2 Ultraviolet

It has been demonstrated that *Arcobacter* can survive wastewater treatment after UV radiation (Webb et al., 2016b), though it should be noted that this report only utilized EMA-qPCR (as opposed to culture) to determine viability, which has been shown to be inconsistent in it's efficacy in determining live vs. dead cells especially in situations with large numbers of dead cells present (Seinige et al., 2014).

3.2.3 Natural processes

No information available

a. Natural sunlight
b. Solar beds (solarization)
c. Dessication

References

Akincioglu, F. (2011). Isolation of arcobacter species from different water sources and characterization of ısolated species by molecular techniques. İzmir Institute of Technology.

Al-Habaty, S.H. and Ammar, M.A.M. (2015). *Arcobacter* Species and Their Risks in Some Meat and Fish With a Sodium Acetate and Sodium Chloride Intervention. Assiut Veterinary Medical Journal. 61, pp. 101–114.

Al-Jassim, N., Ansari, M.Ikram, Harb, M. and Hong, P.Y. (2015). Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: Is the treated wastewater safe to reuse for agricultural irrigation?. Water Research. 73, pp. 277–290.

Assanta, M.A., Roy, D., Lemay, M.J. and Montpetit, D. (2002). Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. Journal of Food Protection. 65, pp. 1240–1247.

Atabay, H.I., Corry, J.E. and On, S.L. (1998). Diversity and prevalence of *Arcobacter spp*. in broiler chickens. Journal of Applied Microbiology. 84, pp. 1007–1016.

Atabay, H.I., Unver, A., Sahin, M., Otlu, S., Elmali, M. and Yaman, H. (2008). Isolation of various *Arcobacter* species from domestic geese (Anser anser). Veterinary Microbiology. 128, pp. 400–405.

Atanassova, V., Kessen, V., Reich, F. and Klein, G. (2008). Incidence of *Arcobacter spp*. in poultry: quantitative and qualitative analysis and PCR differentiation. Journal of Food Protection. 71, pp. 2533–2536.

Bücker, R., Troeger, H., Kleer, J., Fromm, M. and Schulzke, J.D. (2009). *Arcobacter butzleri* induces barrier dysfunction in intestinal HT-29/B6 cells. Journal of Infectious Diseases. 200, pp. 756–764.

Banting, G.S., Braithwaite, S., Scott, C., Kim, J., Jeon, B., Ashbolt, N. *et al*. (2016). Evaluation of Various *Campylobacter*-Specific Quantitative PCR (qPCR) Assays for Detection and Enumeration of *Campylobacteraceae* in Irrigation Water and Wastewater via a Miniaturized Most-Probable-Number-qPCR Assay. Applied and Environmental Microbiology. 82, pp. 4743–4756.

Cai, L., Ju, F. and Zhang, T. (2014). Tracking human sewage microbiome in a municipal wastewater treatment plant. Applied Microbiology and Biotechnology. 98, pp. 3317–3326.

Castro-Hermida, J.A., García-Presedo, I., González-Warleta, M. and Mezo, M. (2010). *Cryptosporidium* and *Giardia* detection in water bodies of Galicia, Spain. Water Research. 44, pp. 5887–5896.

Chinivasagam, H.N., Corney, B.G., Wright, L.L., Diallo, I.S. and Blackall, P.J. (2007). Detection of *Arcobacter spp*. in piggery effluent and effluent-irrigated soils in southeast Queensland. Journal of Applied Microbiology. 103, pp. 418–426.

Coker, A.O., Isokpehi, R.D., Thomas, B.N., Amisu, K.O. and Obi, C.L. (2002). Human campylobacteriosis in developing countries. Emerging Infectious Diseases. 8, pp. 237–244.

Collado, L., Cleenwerck, I., Van Trappen, S., De Vos, P. and Figueras, M.J. (2009). *Arcobacter* mytili sp. nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels. International Journal of Systematic and Evolutionary Microbiology. 59, pp. 1391–1396.

Collado, L. and Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. Clinical Microbiology Reviews. 24, pp. 174–192.

Collado, L., Guarro, J. and Figueras, M.J. (2009). Prevalence of *Arcobacter* in meat and shellfish. Journal of Food Protection. 72, pp. 1102–1106.

Collado, L., Gutiérrez, M., González, M. and Fernández, H. (2013). Assessment of the prevalence and diversity of emergent campylobacteria in human stool samples using a combination of traditional and molecular methods. Diagnostic Microbiology and Infectious Disease. 75, pp. 434–436.

Collado, L., Inza, I., Guarro, J. and Figueras, M.J. (2008). Presence of *Arcobacter spp*. in environmental waters correlates with high levels of fecal pollution. Environmental Microbiology. 10, pp. 1635–1640.

Collado, L., Kasimir, G., Perez, U., Bosch, A., Pinto, R., Saucedo, G. *et al*. (2010). Occurrence and diversity of *Arcobacter spp*. along the Llobregat River catchment, at sewage effluents and in a drinking water treatment plant. Water Research. 44, pp. 3696–3702.

de Boer, R.F., Ott, A., Güren, P., *van* Zanten, E., *van* Belkum, A. and Kooistra-Smid, A.M.D. (2013). Detection of *Campylobacter* species and *Arcobacter butzleri* in stool samples by use of real-time multiplex PCR. Journal of Clinical Microbiology. 51, pp. 253–259.

De Smet, S., De Zutter, L., Debruyne, L., Vangroenweghe, F., Vandamme, P. and Houf, K. (2011). *Arcobacter* population dynamics in pigs on farrow-to-finish farms. Applied and Environmental Microbiology. 77, pp. 1732–1738.

De Smet, S., De Zutter, L., Debruyne, L., Vangroenweghe, F., Vandamme, P. and Houf, K. (2011). *Arcobacter* population dynamics in pigs on farrow-to-finish farms. Applied and Environmental Microbiology. 77, pp. 1732–1738.

De Smet, S., De Zutter, L. and Houf, K. (2012). Spatial distribution of the emerging foodborne pathogen *Arcobacter* in the gastrointestinal tract of pigs. Foodborne Pathogens and Disease. 9, pp. 1097–1103.

Diéguez, A.L., Balboa, S., Magnesen, T. and Romalde, J.L. (2017). *Arcobacter* lekithochrous sp. nov., a new species isolated from a molluscan hatchery in Norway. International Journal of Systematic and Evolutionary Microbiology. 67(5), pp. 1327-1332.

Diergaardt, S.M., Venter, S.N., Spreeth, A., Theron, J. and Brözel, V.S. (2004). The occurrence of campylobacters in water sources in South Africa. Water Research. 38, pp. 2589–2595.

Donachie, S.P., Bowman, J.P., On, S.L.W. and Alam, M. (2005). *Arcobacter halophilus* sp. nov., the first obligate halophile in the genus *Arcobacter*. International Journal of Systematic and Evolutionary Microbiology. 55, pp. 1271–1277.

Douidah, L., De Zutter, L., Baré, J., De Vos, P., Vandamme, P., Vandenberg, O. *et al*. (2012). Occurrence of putative virulence genes in *Arcobacter* species isolated from humans and animals. Journal of Clinical Microbiology. 50, pp. 735–741.

Dumas, M.D., Polson, S.W., Ritter, D., Ravel, J., Gelb, J., Morgan, R. *et al*. (2011). Impacts of poultry house environment on poultry litter bacterial community composition. PLoS ONE. 6, pp. e24785.

Ertas, N., Dogruer, Y., Gonulalan, Z., Guner, A. and Ulger, I. (2010). Prevalence of arcobacter species in drinking water, spring water, and raw milk as determined by multiplex PCR. Journal of Food Protection. 73, pp. 2099–2102.

Fera, M.T., Gugliandolo, C., Lentini, V., Favaloro, A., Bonanno, D., La Camera, E. *et al*. (2010). Specific detection of *Arcobacter spp*. in estuarine waters of Southern Italy by PCR and fluorescent *in situ* hybridization. Letters in Applied Microbiology. 50, pp. 65–70.

Fera, M.T., Maugeri, T.L., Gugliandolo, C., Beninati, C., Giannone, M., La Camera, E. *et al*. (2004). Detection of *Arcobacter spp*. in the coastal environment of the Mediterranean Sea. Applied and Environmental Microbiology. 70, pp. 1271–1276.

Fera, M.T., Maugeri, T.L., Gugliandolo, C., La Camera, E., Lentini, V., Favaloro, A. *et al*. (2008). Induction and resuscitation of viable nonculturable *Arcobacter butzleri* cells. Applied and Environmental Microbiology. 74, pp. 3266–3268.

Fera, M.T., Russo, G.T., Di Benedetto, A., La Camera, E., Orlando, A., Giandalia, A. *et al*. (2010). High prevalence of arcobacter carriage in older subjects with type 2 diabetes. Journal of Biomedicine and Biotechnology. 2010, pp. 489784–7.

Fernández, N., Díaz, E.E., Amils, R. and Sanz, J.L. (2008). Analysis of microbial community during biofilm development in

an anaerobic wastewater treatment reactor. Microbial Ecology. 56, pp. 121–132.

Fernandez-Cassi, X., Silvera, C., Cervero-Aragó, S., Rusinol, M., Latif-Eugeni, F., Bruguera-Casamada, C. *et al*. (2016). Evaluation of the microbiological quality of reclaimed water produced from a lagooning system. Environmental Science and Pollution Research International. 23(16), pp. 1–18.

Fernandez-Cassi, X., Silvera, C., Cervero-Aragó, S., Rusinol, M., Latif-Eugeni, F., Bruguera-Casamada, C. *et al*. (2016). Evaluation of the microbiological quality of reclaimed water produced from a lagooning system. Environmental Science and Pollution Research. pp. 1–18. doi: 10.1007/s11356-016-6812-0.

Ferreira, S., Fraqueza, M.J., Queiroz, J.A., Domingues, F.C. and Oleastro, M. (2013). Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. International Journal of Food Microbiology. 162, pp. 82–88.

Ferreira, S., Júlio, C., Queiroz, J.A., Domingues, F.C. and Oleastro, M. (2014). Molecular diagnosis of *Arcobacter* and *Campylobacter* in diarrhoeal samples among Portuguese patients. Diagnostic Microbiology and Infectious Disease. 78, pp. 220–225.

Ferreira, S., Queiroz, J.A., Oleastro, M. and Domingues, F.C. (2015). Insights in the pathogenesis and resistance of *Arcobacter*: A review. Critical Reviews in Microbiology. pp. 1–20.

Figueras, M.J., Levican, A., Pujol, I., Ballester, F., Rabada-Quilez, M.J. and Gomez-Bertomeu, F. (2014). A severe case of persistent diarrhoea associated with *Arcobacter cryaerophilus* but attributed to *Campylobacter* sp. and a review of the clinical incidence of *Arcobacter spp*. New Microbes and New Infections. 2, pp. 31–37.

Figueras, M.J., Pérez-Cataluña, A., Salas-Massó, N., Levican, A. and Collado, L. (2017). '*Arcobacter* porcinus' sp. nov., a novel *Arcobacter* species uncovered by *Arcobacter thereius*. New Microbes and New Infections. 15, pp. 104–106.

Figueras, M.J., Collado, L. and Guarro, J. (2008). A new 16S rDNA-RFLP method for the discrimination of the accepted species of *Arcobacter*. Diagnostic Microbiology and Infectious Disease. 62, pp. 11–15.

Figueras, M.J., Collado, L., Levican, A., Perez, J., Solsona, M.J. and Yustes, C. (2011). *Arcobacter* molluscorum sp. nov., a new species isolated from shellfish. Systematic and Applied Microbiology. 34, pp. 105–109.

Figueras, M.J., Levican, A. and Collado, L. (2012). Updated 16S rRNA-RFLP method for the identification of all currently characterised *Arcobacter spp*. BMC Microbiology. 12, pp. 292.

Figueras, M.J., Levican, A., Collado, L., Inza, M.I. and Yustes, C. (2011). *Arcobacter* ellisii sp. nov., isolated from mussels. Systematic and Applied Microbiology. 34, pp. 414–418.

Fisher, J.C., Levican, A., Figueras, M.J. and McLellan, S.L. (2014). Population dynamics and ecology of *Arcobacter* in sewage. Frontiers in Microbiology. 5, pp. 525.

Forbes, K.J., Gormley, F.J., Dallas, J.F., Labovitiadi, O., MacRae, M., Owen, R.J. *et al*. (2009). *Campylobacter* immunity and coinfection following a large outbreak in a farming community. Journal of Clinical Microbiology. 47, pp. 111–116.

Giacometti, F., Lucchi, A., Di Francesco, A., Delogu, M., Grilli, E., Guarniero, I. *et al*. (2015). *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* Circulation in a Dairy Farm and Sources of Milk Contamination. Applied and Environmental Microbiology. 81, pp. 5055–5063.

Giacometti, F., Salas-Massó, N., Serraino, A. and Figueras, M.J. (2015). Characterization of *Arcobacter* suis isolated from water buffalo (Bubalus bubalis) milk. Food Microbiology. 51, pp. 186–191.

Gilbert, M.J., Kik, M., Timmerman, A.J., Severs, T.T., Kusters, J.G., Duim, B. *et al*. (2014). Occurrence, diversity, and host association of intestinal *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles. PLoS ONE. 9, pp. e101599.

Girbau, C., Guerra, C., Martínez-Malaxetxebarria, I., Alonso, R. and Fernández-Astorga, A. (2015). Prevalence of ten putative virulence genes in the emerging foodborne pathogen *Arcobacter* isolated from food products. Food Microbiology.

52, pp. 146–149.

González, A., Botella, S., Montes, R.M., Moreno, Y. and Ferrús, M.A. (2007). Direct detection and identification of *Arcobacter* species by multiplex PCR in chicken and wastewater samples from Spain. Journal of Food Protection. 70, pp. 341–347.

González, A. and Ferrús, M.A. (2011). Study of *Arcobacter spp*. contamination in fresh lettuces detected by different cultural and molecular methods. International Journal of Food Microbiology. 145, pp. 311–314.

González, A., Suski, J. and Ferrús, M.A. (2010). Rapid and accurate detection of *Arcobacter* contamination in commercial chicken products and wastewater samples by real-time polymerase chain reaction. Foodborne Pathogens and Disease. 7, pp. 327–338.

Gonzalez-Martinez, A., Rodriguez-Sanchez, A., Lotti, T., Garcia-Ruiz, M.-J., Osorio, F., Gonzalez-Lopez, J. *et al*. (2016). Comparison of bacterial communities of conventional and A-stage activated sludge systems. Scientific Reports. 6, pp. 18786.

L Gras, M., Smid, J.H., Wagenaar, J.A., Koene, M.G.J., Havelaar, A.H., Friesema, I.H.M. *et al*. (2013). Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. Epidemiology and Infection. 141, pp. 2526–2535.

Grove-White, D.H., Leatherbarrow, A.J.H., Cripps, P.J., Diggle, P.J. and French, N.P. (2014). Temporal and farm-management-associated variation in faecal pat prevalence of *Arcobacter spp*. in ruminants. Epidemiology and Infection. 142, pp. 861–870.

Hakim, J.A., Koo, H., Dennis, L.N., Kumar, R., Ptacek, T., Morrow, C.D. *et al*. (2015). An abundance of Epsilonproteobacteria revealed in the gut microbiome of the laboratory cultured sea urchin, *Lytechinus variegatus*. Frontiers in Microbiology. 6, pp. 1047.

Hamann, E., Gruber-Vodicka, H., Kleiner, M., Tegetmeyer, H.E., Riedel, D., Littmann, S. *et al*. (2016). Environmental Breviatea harbour mutualistic *Arcobacter* epibionts. Nature. 534, pp. 254–258.

Hausdorf, L., Neumann, M., Bergmann, I., Sobiella, K., Mundt, K., Fröhling, A. *et al*. (2013). Occurrence and genetic diversity of *Arcobacter spp*. in a spinach-processing plant and evaluation of two *Arcobacter*-specific quantitative PCR assays. Systematic and Applied Microbiology. 36, pp. 235–243.

He, Y., Xie, K., Xu, P., Huang, X., Gu, W., Zhang, F. *et al*. (2013). Evolution of microbial community diversity and enzymatic activity during composting. Research in Microbiology. 164, pp. 189–198.

Higgins, R., Messier, S., Daignault, D. and Lorange, M. (1999). *Arcobacter butzleri* isolated from a diarrhoeic non-human primate. Laboratory Animals. 33, pp. 87–90.

Ho, H.T.K., Lipman, L.J.A. and Gaastra, W. (2008). The introduction of *Arcobacter spp*. in poultry slaughterhouses. International Journal of Food Microbiology. 125, pp. 223–229.

Ho, T.K.H., Lipman, L.J.A., *van* der Graaf-*van* Bloois, L., *van* Bergen, M. and Gaastra, W. (2006). Potential routes of acquisition of *Arcobacter* species by piglets. Veterinary Microbiology. 114, pp. 123–133.

Houf, K., De Smet, S., Baré, J. and Daminet, S. (2008). Dogs as carriers of the emerging pathogen *Arcobacter*. Veterinary Microbiology. 130, pp. 208–213.

Houf, K., De Zutter, L., *van* Hoof, J. and Vandamme, P. (2002). Occurrence and distribution of *Arcobacter* species in poultry processing. Journal of Food Protection. 65, pp. 1233–1239.

Houf, K., On, S.L.W., Coenye, T., Debruyne, L., De Smet, S. and Vandamme, P. (2009). *Arcobacter thereius* sp. nov., isolated from pigs and ducks. International Journal of Systematic and Evolutionary Microbiology. 59, pp. 2599–2604.

Houf, K. and Stephan, R. (2007). Isolation and characterization of the emerging foodborn pathogen *Arcobacter* from human

stool. Journal of Microbiological Methods. 68, pp. 408–413.

Houf, K., Tutenel, A., Zutter, L., Hoof, J. and Vandamme, P. (2000). Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiology Letters. 193, pp. 89–94.

Hsu, T.-.T.D. and Lee, J. (2015). Global Distribution and Prevalence of *Arcobacter* in Food and Water. Zoonoses and Public Health. 62(8), pp. 579-589.

G Inglis, D., McAllister, T.A., Larney, F.J. and Topp, E. (2010). Prolonged survival of *Campylobacter* species in bovine manure compost. Applied and Environmental Microbiology. 76, pp. 1110–1119.

Jacob, J., Lior, H. and Feuerpfeil, I. (1993). Isolation of *Arcobacter butzleri* from a drinking water reservoir in eastern Germany. Microbial Ecology. 193(6), pp. 121–132.

Jacob, J., Woodward, D., Feuerpfeil, I. and Johnson, W.M. (1998). Isolation of *Arcobacter butzleri* in raw water and drinking water treatment plants in Germany. Zentralblatt fur Hygiene und Umweltmedizin= International Journal of Hygiene and Environmental Medicine. 201, pp. 189–198.

Jalava, K., Rintala, H., Ollgren, J., Maunula, L., Gomez-Alvarez, V., Revez, J. *et al*. (2014). Novel microbiological and spatial statistical methods to improve strength of epidemiological evidence in a community-wide waterborne outbreak. PLoS ONE. 9, pp. e104713.

Jiang, Z.D., DuPont, H.L., Brown, E.L., Nandy, R.K., Ramamurthy, T., Sinha, A. *et al*. (2010). Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and *Arcobacter* species. Journal of Clinical Microbiology. 48, pp. 1417–1419.

Karadas, G., Bücker, R., Sharbati, S., Schulzke, J.D., Alter, T. and Gölz, G. (2016). *Arcobacter butzleri* isolates exhibit pathogenic potential in intestinal epithelial cell models. Journal of Applied Microbiology. 120, pp. 218–225.

Kim, H.M., Hwang, C.Y. and Cho, B.C. (2010). *Arcobacter marinus* sp. nov. International Journal of Systematic and Evolutionary Microbiology. 60, pp. 531–536.

Kjeldgaard, J., Jørgensen, K. and Ingmer, H. (2009). Growth and survival at chiller temperatures of *Arcobacter butzleri*. International Journal of Food Microbiology. 131, pp. 256–259.

Kopilović, B., Ucakar, V., Koren, N., Krek, M. and Kraigher, A. (2008). Waterborne outbreak of acute gastroenteritis in a costal area in Slovenia in June and July 2008. Euro Surveillance : Bulletin Européen sur les Maladies Transmissibles = European Communicable Disease Bulletin. 13(34), .

Laishram, M., Rathlavath, S., Lekshmi, M., Kumar, S. and Nayak, B.B. (2016). Isolation and characterization of *Arcobacter spp*. from fresh seafood and the aquatic environment. International Journal of Food Microbiology. 232, pp. 87–89.

Lappi, V., Archer, J.R., Cebelinski, E., Leano, F., Besser, J.M., Klos, R.F. *et al*. (2013). An Outbreak of Foodborne Illness Among Attendees of a Wedding Reception in Wisconsin Likely Caused by *Arcobacter butzleri*. Foodborne Pathogens and Disease. 10, pp. 250–255.

Lastovica, A.J. and le Roux, E. (2000). Efficient isolation of campylobacteria from stools. Journal of Clinical Microbiology. 38, pp. 2798–2799.

Lee, C., Agidi, S., Marion, J.W. and Lee, J. (2012). *Arcobacter* in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. Applied and Environmental Microbiology. 78, pp. 5511–5519.

Leoni, F., Chierichetti, S., Santarelli, S., Talevi, G., Masini, L., Bartolini, C. *et al*. (2017). Occurrence of *Arcobacter spp*. and correlation with the bacterial indicator of faecal contamination *Escherichia coli* in bivalve molluscs from the Central Adriatic, Italy. International Journal of Food Microbiology. 245, pp. 6–12.

Levican, A., Alkeskas, A., Günter, C., Forsythe, S.J. and Figueras, M.J. (2013). Adherence to and invasion of human

intestinal cells by *Arcobacter* species and their virulence genotypes. Applied and Environmental Microbiology. 79, pp. 4951–4957.

Levican, A., Collado, L., Aguilar, C., Yustes, C., Diéguez, A.L., Romalde, J.L. *et al*. (2012). *Arcobacter* bivalviorum sp. nov. and *Arcobacter* venerupis sp. nov., new species isolated from shellfish. Systematic and Applied Microbiology. 35, pp. 133–138.

Levican, A., Collado, L. and Figueras, M.J. (2016). The use of two culturing methods in parallel reveals a high prevalence and diversity of *Arcobacter spp*. in a wastewater treatment plant. BioMed Research International. 2016,.

Levican, A., Collado, L. and Figueras, M.J. (2013). *Arcobacter* cloacae sp. nov. and *Arcobacter* suis sp. nov., two new species isolated from food and sewage. Systematic and Applied Microbiology. 36, pp. 22–27.

Levican, A., Collado, L., Yustes, C., Aguilar, C. and Figueras, M.J. (2014). Higher water temperature and incubation under aerobic and microaerobic conditions increase the recovery and diversity of *Arcobacter spp*. from shellfish. Applied and Environmental Microbiology. 80, pp. 385–391.

Levican, A. and Figueras, M.J. (2013). Performance of five molecular methods for monitoring *Arcobacter spp*. BMC Microbiology. 13, pp. 220.

Levican, A., Rubio-Arcos, S., Martinez-Murcia, A., Collado, L. and Figueras, M.J. (2015). *Arcobacter* ebronensis sp. nov. and *Arcobacter* aquimarinus sp. nov., two new species isolated from marine environment. Systematic and Applied Microbiology. 38, pp. 30–35.

Lipman, L., Ho, H. and Gaastra, W. (2008). The presence of *Arcobacter* species in breeding hens and eggs from these hens. Poultry Science. 87, pp. 2404–2407.

Mandisodza, O., Burrows, E. and Nulsen, M. (2012). *Arcobacter* species in diarrhoeal faeces from humans in New Zealand. The New Zealand Medical Journal (Online). 125(1353), pp. 40-46.

Maugeri, T.L., Carbone, M., Fera, M.T., Irrera, G.P. and Gugliandolo, C. (2004). Distribution of potentially pathogenic bacteria as free living and plankton associated in a marine coastal zone. Journal of Applied Microbiology. 97, pp. 354–361.

McLellan, S.L., Huse, S.M., Mueller-Spitz, S.R., Andreishcheva, E.N. and Sogin, M.L. (2010). Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. Environmental Microbiology. 12, pp. 378–392.

Merga, J.Y., Leatherbarrow, A.J.H., Winstanley, C., Bennett, M., Hart, C.A., Miller, W.G. *et al*. (2011). Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter spp*. in Cheshire, United Kingdom. Applied and Environmental Microbiology. 77, pp. 1646–1650.

Merga, J.Y., Royden, A., Pandey, A.K. and Williams, N.J. (2014). *Arcobacter spp*. isolated from untreated domestic effluent. Letters in Applied Microbiology. 59, pp. 122–126.

Miller, W.G., Parker, C.T., Rubenfield, M., Mendz, G.L., Wösten, M.M.S.M., Ussery, D.W. *et al*. (2007). The complete genome sequence and analysis of the epsilonproteobacterium *Arcobacter butzleri*. PLoS ONE. 2, pp. e1358.

Moreno, Y., Botella, S., Alonso, J.L., Ferrús, M.A., Hernández, M. and Hernández, J. (2003). Specific detection of *Arcobacter* and *Campylobacter* strains in water and sewage by PCR and fluorescent *in situ* hybridization. Applied and Environmental Microbiology. 69, pp. 1181–1186.

Morita, Y., Maruyama, S., Kabeya, H., Boonmar, S., Nimsuphan, B., Nagai, A. *et al*. (2004). Isolation and phylogenetic analysis of arcobacter spp. in ground chicken meat and environmental water in Japan and Thailand. Microbiology and Immunology. 48, pp. 527–533.

Neill, S.D., Ellis, W.A. and O'Brien, J.J. (1978). The biochemical characteristics of *Campylobacter*-like organisms from cattle and pigs. Research in Veterinary Science. 25, pp. 368–372.

Newton, R.J., Bootsma, M.J., Morrison, H.G., Sogin, M.L. and McLellan, S.L. (2013). A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. Microbial Ecology. 65, pp. 1011–1023.

Ottaviani, D., Chierichetti, S., Rocchegiani, E., Bartolini, C., Masini, L., Santarelli, S. *et al*. (2013). Bioaccumulation experiments in mussels contaminated with the food-borne pathogen *Arcobacter butzleri*: preliminary data for risk assessment. BioMed Research International. 2013(2), pp. 153419.

Park, E., Mancl, K.M., Tuovinen, O.H., Bisesi, M.S. and Lee, J. (2016). Ensuring safe reuse of residential wastewater: reduction of microbes and genes using peat biofilter and batch chlorination in an on-site treatment system. Journal of Applied Microbiology. 121, pp. 1777–1788.

Patyal, A., Rathore, R.S., Mohan, H.V., Dhama, K. and Kumar, A. (2011). Prevalence of *Arcobacter spp*. in humans, animals and foods of animal origin including sea food from India. Transboundary and Emerging Diseases. 58, pp. 402–410.

Piva, S., Gariano, G.R., Bonilauri, P., Giacometti, F., Decastelli, L., Florio, D. *et al*. (2017). Occurrence of putative virulence genes on *Arcobacter butzleri* isolated from three different environmental sites throughout the dairy chain. Journal of Applied Microbiology. 122, pp. 1071–1077.

Rahimi, E. (2014). Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. British Poultry Science. 55, pp. 174–180.

Rea, C.L., Bisesi, M.S., Mitsch, W., Andridge, R. and Lee, J. (2015). Human health-related ecosystem services of avian-dense coastal wetlands adjacent to a Western Lake Erie swimming beach. EcoHealth. 12, pp. 77–87.

Revez, J., Huuskonen, M., Ruusunen, M., Lindström, M. and Hänninen, M.L. (2013). *Arcobacter* species and their pulsed-field gel electrophoresis genotypes in Finnish raw milk during summer 2011. Journal of Food Protection. 76, pp. 1630–1632.

Rice, E.W., Rodgers, M.R., Wesley, I.V., Johnson, C.H. and Tanner, S.A. (1999). Isolation of *Arcobacter butzleri* from ground water. Letters in Applied Microbiology. 28, pp. 31–35.

Salas-Massó, N., Andree, K.B., Furones, M.D. and Figueras, M.J. (2016). Enhanced recovery of *Arcobacter spp*. using NaCl in culture media and re-assessment of the traits of *Arcobacter marinus* and *Arcobacter halophilus* isolated from marine water and shellfish. The Science of the Total Environment. 566, pp. 1355-1361.

Samie, A., Obi, C.L., Barrett, L.J., Powell, S.M. and Guerrant, R.L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: studies using molecular diagnostic methods. The Journal of Infection. 54, pp. 558–566.

Sasi Jyothsna, T.S., Rahul, K., Ramaprasad, E.V.V., Sasikala, C. and Ramana, C.V. (2013). *Arcobacter* anaerophilus sp. nov., isolated from an estuarine sediment and emended description of the genus *Arcobacter*. International Journal of Systematic and Evolutionary Microbiology. 63, pp. 4619–4625.

Scarano, C., Giacometti, F., Manfreda, G., Lucchi, A., Pes, E., Spanu, C. *et al*. (2014). *Arcobacter butzleri* in sheep ricotta cheese at retail and related sources of contamination in an industrial dairy plant. Applied and Environmental Microbiology. 80, pp. 7036–7041.

Seinige, D., Krischek, C., Klein, G. and Kehrenberg, C. (2014). Comparative analysis and limitations of ethidium monoazide and propidium monoazide treatments for the differentiation of viable and nonviable *Campylobacter* cells. Applied and Environmental Microbiology. 80, pp. 2186–2192.

Serraino, A., Florio, D., Giacometti, F., Piva, S., Mion, D. and Zanoni, R.G. (2013). Presence of *Campylobacter* and *Arcobacter* species in in-line milk filters of farms authorized to produce and sell raw milk and of a water buffalo dairy farm in Italy. Journal of Dairy Science. 96, pp. 2801–2807.

Serraino, A., Giacometti, F., Daminelli, P., Losio, M.N., Finazzi, G., Marchetti, G. *et al*. (2013). Survival of *Arcobacter butzleri* during production and storage of artisan water buffalo mozzarella cheese. Foodborne Pathogens and Disease. 10, pp. 820–824.

Shah, A.H., Saleha, A.A., Zunita, Z., Murugaiyah, M., Aliyu, A.B. and Jafri, N. (2013). Prevalence, distribution and antibiotic

resistance of emergent *Arcobacter spp*. from clinically healthy cattle and goats. Transboundary and Emerging Diseases. 60, pp. 9–16.

Shanks, O.C., Newton, R.J., Kelty, C.A., Huse, S.M., Sogin, M.L. and McLellan, S.L. (2013). Comparison of the microbial community structures of untreated wastewaters from different geographic locales. Applied and Environmental Microbiology. 79, pp. 2906–2913.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J. and Harrison, M.A. (2007). Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. International Journal of Food Microbiology. 113, pp. 16–22.

Stampi, S., Varoli, O., Zanetti, F. and De Luca, G. (1993). *Arcobacter cryaerophilus* and thermophilic campylobacters in a sewage treatment plant in Italy: two secondary treatments compared. Epidemiology and Infection. 110, pp. 633–639.

Stampi, S., De Luca, G., Varoli, O. and Zanetti, F. (1999). Occurrence, Removal and Seasonal Variation of Thermophilic Campylobacters and *Arcobacter* in Sewage Sludge. Zentralblatt für Hygiene und Umweltmedizin = International Journal of Hygiene and Environmental Medicine. 202, pp. 19–27.

Strachan, N.J.C., Rotariu, O., Smith-Palmer, A., Cowden, J., Sheppard, S.K., O'Brien, S.J. *et al*. (2013). Identifying the seasonal origins of human campylobacteriosis. Epidemiology and Infection. 141, pp. 1267–1275.

Strauman, M.C., Harper, J.M., Harrington, S.M., Boll, E.J. and Nataro, J.P. (2010). Enteroaggregative *Escherichia coli* disrupts epithelial cell tight junctions. Infection and Immunity. 78, pp. 4958–4964.

Suh, S.S., Park, M., Hwang, J., Kil, E.J., Jung, S.W., Lee, S. *et al*. (2015). Seasonal Dynamics of Marine Microbial Community in the South Sea of Korea. PLoS ONE. 10, pp. e0131633.

Tomaras, J., Sahl, J.W., Siegrist, R.L. and Spear, J.R. (2009). Microbial diversity of septic tank effluent and a soil biomat. Applied and Environmental Microbiology. 75, pp. 3348–3351.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. *et al*. (1991). Revision of *Campylobacter*, *Helicobacter*, and Wolinella taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. International Journal of Systematic Bacteriology. 41, pp. 88–103.

Vandamme, P., Pugina, P., Benzi, G., Van Etterijck, R., Vlaes, L., Kersters, K. *et al*. (1992). Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. Journal of Clinical Microbiology. 30, pp. 2335–2337.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D. *et al*. (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. International Journal of Systematic Bacteriology. 42, pp. 344–356.

Van den Abeele, A.M., Vogelaers, D., Van Hende, J. and Houf, K. (2014). Prevalence of *Arcobacter* species among humans, Belgium, 2008-2013. Emerging Infectious Diseases. 20, pp. 1731–1734.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S. *et al*. (2004). *Arcobacter* species in humans. Emerging Infectious Diseases. 10, pp. 1863–1867.

Vandewalle, J.L., Goetz, G.W., Huse, S.M., Morrison, H.G., Sogin, M.L., Hoffmann, R.G. *et al*. (2012). *Acinetobacter*, *Aeromonas* and Trichococcus populations dominate the microbial community within urban sewer infrastructure. Environmental Microbiology. 14, pp. 2538–2552.

Van Driessche, E. and Houf, K. (2008). Survival capacity in water of *Arcobacter* species under different temperature conditions. Journal of Applied Microbiology. 105, pp. 443–451.

Van Driessche, E. and Houf, K. (2007). Discrepancy between the occurrence of *Arcobacter* in chickens and broiler carcass contamination. Poultry Science. 86, pp. 744–751.

Van Driessche, E., Houf, K., Vangroenweghe, F., De Zutter, L. and *van* Hoof, J. (2005). Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. Veterinary Microbiology. 105, pp. 149–154.

Van Driessche, E., Houf, K., Vangroenweghe, F., Nollet, N., De Zutter, L., Vandamme, P. *et al*. (2004). Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. Research in Microbiology. 155, pp. 662–666.

Van Driessche, E., Houf, K., *van* Hoof, J., De Zutter, L. and Vandamme, P. (2003). Isolation of *Arcobacter* species from animal feces. FEMS Microbiology Letters. 229, pp. 243–248.

VanDyke, M.I., Morton, V.K., McLellan, N.L. and Huck, P.M. (2010). The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada. Journal of Applied Microbiology. 109, pp. 1053-1066.

Webb, A.L., Boras, V.F., Kruczkiewicz, P., Selinger, L.B., Taboada, E.N. and Inglis, G.D. (2016). Comparative detection and quantification of *Arcobacter butzleri* in stools from diarrheic and non-diarrheic human beings in southwestern Alberta, Canada. Journal of Clinical Microbiology. 54(4), pp. 1082-1088.

Webb, A.L., Taboada, E.N., L Selinger, B., Boras, V.F. and G Inglis, D. (2016). Efficacy of wastewater treatment on *Arcobacter butzleri* density and strain diversity. Water Research. 105, pp. 291–296.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. *et al*. (2000). Fecal shedding of *Campylobacter* and *Arcobacter spp*. in dairy cattle. Applied and Environmental Microbiology. 66, pp. 1994–2000.

Wesley, I.V. and Schroeder-Tucker, L. (2011). Recovery of *Arcobacter spp*. from nonlivestock species. Journal of Zoo and Wildlife Medicine. 42, pp. 508–512.

Whiteduck-Léveillée, J., Lapen, D.R., Whiteduck-Léveillée, K., Tambong, J.T., C Lévesque, A., Cloutier, M. *et al*. (2015). *Arcobacter lanthieri* sp. nov., isolated from pig and dairy cattle manure. International Journal of Systematic and Evolutionary Microbiology. 65, pp. 2709–2716.

Whiteduck-Léveillée, K., Whiteduck-Léveillée, J., Cloutier, M., Tambong, J.T., Xu, R., Topp, E. *et al*. (2015). Identification, characterization and description of *Arcobacter faecis* sp. nov., isolated from a human waste septic tank. Systematic and Applied Microbiology. 39(2), pp. 93-99.

Yang, B., Kong, X., Cui, B., Jin, D., Deng, Y., Zhuang, X. *et al*. (2015). Impact of Rural Domestic Wastewater Irrigation on the Physicochemical and Microbiological Properties of Pakchoi and Soil. Water. 7, pp. 1825–1839.

Yesilmen, S., Vural, A., Erkan, M.E. and Yildirim, I.H. (2014). Prevalence and antimicrobial susceptibility of *Arcobacter* species in cow milk, water buffalo milk and fresh village cheese. International Journal of Food Microbiology. 188, pp. 11–14.

Zhang, Z., Yu, C., Wang, X., Yu, S. and Zhang, X.H. (2015). *Arcobacter* pacificus sp. nov., isolated from seawater of the South Pacific Gyre. International Journal of Systematic and Evolutionary Microbiology. 66(2), pp. 542-547.