Review Article

Occurrence of Emerging *Arcobacter* in Dogs and Cats and its Public Health Implications: A Review

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Abstract | *Arcobacter* has emerged as one of the leading cause of gastro-enteritis in humans as well as animals, therefore posing a significant public health risk. The most important of the species in associated with human and animal infection is *A. butzleri.* This is because of the emergence of highly pathogenic and multi-drug resistant *Arcobacter* strains worldwide. Dogs and cats are considered as a major source of transmission to man, thus facilitating easy transmission of the *Arcobacter* infection. Stray dogs and cats are the important reservoirs compared to pets which are also implicated in the transmission to humans. Molecular techniques such as Polymerase chain reaction (PCR), Pulsed-field gel electrophoresis (PFGE) and Multi-locus Sequence Typing Scheme (MLST) has been found to be one of the most robust, accurate and sensitive technique for the detection and characterization of *Arcobacter* species in dogs and cats. This review focuses on the occurrence and associated risk factors as well as public health significance of *Arcobacter* in dogs and cats.

Keywords | *Arcobacter*, Dogs, Cats, Public health significance, Antibiotic resistance

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INTRODUCTION

Arcobacter is widely regarded as an emerging food-borne pathogen because of its relationship with food production and human health. *Arcobacter* was initially recognized as 'aerotolerant *Campylobacter*' belonging to the family *Campylobacteraceae*, genus *Campylobacter* due to its phenotypic and phylogenetic resemblance with *Campylobacter*. However, the ability to grow at 15°C and its aero tolerance distinguishes it from *Campylobacter* (Collado and Figueras, 2011). From the discovery of *Arcobacter* in 1977 to date, various species have been identified and discovered in various animals which include domestic animals, pets, wild animals, birds and food products originating from domestic animals. These may result in diseases such as mastitis, abortion and diarrhoea in animals (Merga et al., 2011). Several species have so far been identified of which some are regarded as emerging food-borne pathogens namely: *A. butzleri*, *A. skirrowii*, *A. cryaerophilus*, *A. cibarius*, *A. mytili*, *A. thereius*, and *A. trophiarum*. *Arcobacter butzleri*,

A. skirrowii, and *A. cryaerophilus* have been isolated from faecal samples of human beings and healthy farm animals (Driessche et al., 2005; Merga et al., 2011). However, the current identification and detection method of *Arcobacter* species is difficult and cumbersome therefore the incidence is most likely underestimated (Vandenberg et al., 2004).

Arcobacter was first discovered when it was isolated from aborted bovine foetuses in UK in 1977 (Ellis et al., 1977). The members of this genus were initially named as *Campylobacter cryaerophila* due to the similarities in morphology, aero tolerance and growth at 25°C to the genus *Campylobacter*. The species of the *Campylobacter* that are considered as aero tolerant species were later re-classified into the genus *Arcobacter* (Collado and Figueras, 2011). They are gram-negative spiral-shaped organisms and have the ability to grow under microaerobic or aerobic conditions (Lehner et al., 2005; Vandenberg et al., 2004).

Arcobacter is both a food-borne and water-borne agent and is an organism considered to be one of the most leading emerging zoonotic pathogen (Houf et al., 2004). Among the species currently identified as members of the genus *Arcobacter*, seven species namely *A. butzleri*, *A. skirrowii*, *A. cryaerophilus*, *A. cibarius*, *A. mytili*, *A. thereius* and *A. trophiarum* are considered to be potential emerging food borne pathogens because they had been isolated from environmental niches, shellfish, poultry and faecal materials of domestic animals (Vogelaers et al., 2014; Whiteduck-Léveillée et al., 2015). *Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii* are the common species of *Arcobacter* isolated from human and domestic animals (Gude et al., 2005; Morita et al., 2004; Rahimi, 2014). The rate of isolation of *Arcobacter* species is more numerous in poultry than dogs, cats and other animals which indicates poultry as the primary source of the organism (Amare et al., 2011; Ferreira et al., 2013; Shah et al., 2012; Wesley et al., 1995). It is shown to be occur more frequent in stray dogs and cats than those that are used as pets (Talay et al., 2016).

Arcobacter transmission is usually through the consumption of contaminated food and drinking water; infection can also occur through direct contact with infected animals and humans (Ellis et al., 1977). The organism is ubiquitous and can be found in sewage, surface water, sea water, ground water and drinking water which suggests their wider presence in the environment which can serve as an alternative means of exposure and transmission of infection to animals and humans (Houf et al., 2004; Lehner et al., 2005; Whiteduck-Léveillée et al., 2015). There are a substantial number of studies on the epidemiology of *Arcobacter* globally, but these are limited to livestock animals and not much has been done on their presence in dogs and cats. However, recent studies have shown there occurrence in samples from wastewater and marine environment (Morita et al., 2004; Vogelaers et al., 2014).

Arcobacter infection in humans can be due to exposure and frequent contact with infected dogs and cats. This infection in man is reported to be due to pet ownership and has been identified and reported as a risk factor for its transmission to humans (Rahimi, 2014). The increase in number of dogs and cats kept as pets may thus lead to the increase in *Arcobacter* infection in human due to close contact with these pets (Gude et al., 2004). The frequent use of antibiotics in domestic animals has been widely reported to be the major cause antibiotic resistance development in *Arcobacter*. In pet animals, this problem is of public health significance due to close contact with human resulting in the emergence of antibiotic resistant organisms (Ferreira et al., 2013).

Arcobacter butzleri and *A. cryaerophilus* were detected from the buccal cavity of pet dogs using molecular techniques (Talay et al., 2016). *Arcobacter* have been isolated in oral samples of cats and dogs with oral and dental disease in Denmark (Petersen et al., 2007), however in a study in Belgium *Arcobacter* spp was not isolated from buccal and rectal swabs of pet cats (Houf et al., 2008). In 2008, *Arcobacter* were isolated from oral smears and buccal cavity of dogs and cats, but another study in conducted in on clinical materials from dogs and cats in Southern Italy showed the presence of *Arcobacter* species (Fernandez et al., 2004; Houf et al., 2009; Kim et al., 2010).

Phenotypic, Biochemical Properties And Identification

Phenotypically on blood agar, the colonies appear as pin points, translucent and watery colonies. The organisms are Gram negative when stained with Gram stain, however they exhibit a characteristic cork-screw type of motility (Fera et al., 2004). *Arcobacter* species are relatively similar and related to *Campylobacter* species due to the fact that they cannot ferment carbohydrate but they hydrolyse indoxyl acetate (Fera et al., 2009). They are also known to be relatively biochemically inert and only a few phenotypic tests, including the Preston identification scheme and catalase test can be used to differentiate *Arcobacter* spp. (Moreno et al., 2003). Species identification of *Arcobacter* using standard biochemical tests is unreliable due to their fastidious growth requirements and low metabolic activity usually observed within the *Proteobacteria* together (Ellis et al., 1977; Hausdorf et al., 2013). Therefore to differentiate the species on this basis, a combination of a wide range of biochemical tests with a high tendency of comparing the characteristic of the unknown isolate and those in well-defined taxa is required. *Arcobacter butzleri* can be reliably tested and identified when grown in 1% glycine and in 1.5% NaCl, weak catalase activity, and resistance to cadmium chloride (Gonzalez and Ferrus, 2011; Pejchalova et al., 2016). Table 1 shows different phenotypic and biochemical properties of *Arcobacter* species.

Table 1: Phenotypic and biochemical properties of *Arcobacter* species.

Characteristic	Arcobacter butzleri	Arcobacter skirrowii	Arcobacter cryaerophilus	Arcobacter cibarius
Growth at 25^{0}C	+	+	+	V
Microaerobic conditions at 37^{0}C	+	+	V	+
2% (w/v) NaCl	V	+	V	-
4% (w/v) NaCl	-	+	-	-
Growth on MacConkey agar	+	-	V	+
Growth on Minimal medium	+	-	+	+
Catalase	V	+	+	V
Oxidase	+	+	+	+
Urease	-	-	-	-
Nitrate reduction	+	+	V	-
Indole acetate hydrolysis	+	+	+	+
Ceforperazone resistance (64mg/L)	+	+	+	+
G+C content (mol %)	29-31	29-30	28-29	26.8-27.3

+: ≥95% strains positive; **-:** ≤11% strains positive; **V:** 12-94% strains positive; **Sources:** Collado et al., 2011; Houf et al., 2009

Various methods are available for the isolation of *Arcobacter* via culture, however here again there is no standard method of isolation. In 1977, the first case of *Arcobacter* was reported from aborted livestock fetuses using EMJH P-80 (Ellinghausen-McCullogh-Johnson-Harris Polysorbate), which h is a medium for *Leptospira* growth (Shah et al., 2012). Subsequently, various methods have been put in place for the isolation via enrichment and plating through different protocols that were developed for isolation of *Arcobacter* from several sources (Atabay et al., 1998; Diergaardt et al., 2004; Pejchalova et al., 2016; Wegener 2012).

The culture methods are usually divided into two stages for the detection of *Arcobacter* species; they are the enrichment and the plating stages. The enrichment is mostly done at a temperature of 30°C or below and in the plating procedure, samples are inoculated onto the surface of an agar or inoculated into a semi-solid media. The isolation method may take up to 4 to 5 days at 37°C (Amare et al., 2011). *Arcobacter* broth (AB) supplemented with cefoperazone, amphotericin B, teichoplanin (CAT) as developed by Atabay and Corry (1998) is one of the most common protocols used for the isolation. This is a favourable media for the isolation of the common *Arcobacter* species and it inhibits the growth of *Campylobacter* (Son, 2005). Similar to *Campylobacter*, this involves filtration of the broth through 0.45 µm pore sized cellulose triacetate membrane filter.

Even with all the advances in the various isolation protocols described to date, they are still not regarded as ideal in the determination of true incidence rates and distribution of the species in food and biological samples as some species of *Arcobacter* are inhibited during recovery when certain types of antibiotics are used (Johnson and Murano, 1999; Schroeder-Tucker et al., 1996).

Molecular Detection

The detection and identification of *Arcobacter* species can be achieved through few biochemical tests, therefore, the species of *Arcobacter* are most reliably identified through molecular techniques (On, 1996). However, these techniques are often difficult and cumbersome. Therefore, to overcome these challenges and to allow confirmation of the concurrent presence of different *Arcobacter* species, multiplex PCR (m-PCR) methods have been used for screening enrichment samples prior to isolation (Ellis et al., 1977; Kayman et al., 2012).

PCR assay for the specific detection of *Arcobacter* species is either through purified DNA or crude bacterial cell lysate in which results is obtained in less than 8 hours as compared to several days done using the culture methods. DNA-based assays used for the identification of *Arcobacter* species, are more rapid and have a higher specificity than conventional identification methods (Houf et al., 2001). Different PCR Assay based on oligonucleotide DNA probes have already proved to be valuable tools for diagnostic identification and characterization of *Arcobacter* strains. These techniques rely on the use of gene fragments such as 16S rRNA or 23S rRNA specific for *Arcobacter* species and *A. butzleri* (Kiehlbauch et al., 1991; Scullion et al., 2004). The specificity of the detection of *Arcobacter* by PCR is highly dependent on the specificity of the primer set used. This is due to the relationship of 16S rRNA which are typical mosaic structure of conserved and variable regions, PCR primers can be designed complementary to intervening variable regions allowing the detection and identification of specific groups of micro-organisms (Houf and Stephan, 2007).

Several studies were conducted on the detection of *Arco-*

bacter using the multiplex PCR and were shown to be rapid and specific alternative to the simultaneous detection of different species of *Arcobacter* (Fera et al., 2009). This is useful in large-scale surveys to assess the prevalence and thus in determining the clinical and zoonotic potentiality of *Arcobacter* as well as food quality monitoring (Çelik and Ünver, 2015). In a recent study, mPCR was found more efficient with over all detection level of 18.13%, highly specific, sensitive and time saving for detection and confirmation of *Arcobacter* spp. as compared to conventional cultural methods which revealed over all detection level only 10.20% (Hurtado and Owen, 1997). Similarly, Real Time PCR, PCR-denaturing gradient gel electrophoresis (PCR-DGGE), DNA microarray assay and Matrix-associated laser desorption/ionization-time of flight (MALDI-TOF) have been shown to be useful techniques for different genetic analysis (Alispahic et al., 2010; Brightwell et al., 2007; Petersen et al., 2007; Quinones et al., 2007).

However, Pulsed-field gel electrophoresis (PFGE) and Multi-locus Sequence Typing Scheme (MLST) were also used for characterization of *Arcobacter* (Hyytia-Trees et al., 2007; Miller et al., 2009) The diverse genetic characteristics of *Arcobacter* isolates from assessed the potential use of PFGE for epidemiological surveillance and monitoring during outbreaks. PFGE can used together, with single enzyme amplified fragment length polymorphism (s-AFLP) for characterizing taxonomic and epidemiological relationship among *Arcobacter* and *Campylobacter* (Gonzalez et al., 2007). Another method for the detection of *Arcobacter* species is the Fluorescent *in situ* hybridization (FISH) which gives impressive results due to its rapid and sensitive design. Furthermore, FISH also allows determination of morphological characteristics of microbes, size and cellular rRNA content (Fera et al., 2009; Moreno et al., 2003).

A protocol for the molecular detection using real-time PCR was developed by Abdelbaqi et al. (2007) (Gonzalez et al., 2000). The *gyrA* sequences of *A. butzleri* strains and CCUG 34397 B were aligned with those of the *A. cryaerophilus* strain, the *A. cibarius* CCUG 48482 type strain, the *A. skirrowii* 449/80 type strain, and the *A. nitrofigilis* A169/B type strain by using multiple sequence alignment with hierarchical clustering. Primers (F-Arco-FRET5 and R-Arco-FRET5) were designed using using web Primer3 software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi) to target a conserved region outside the quinolone resistance-determining region. These primers used resulted in the amplification of a 905-bp PCR product. PCR was performed with PWO super yield *Taq* polymerase (Roche Diagnostics, Meylan, France). The expected sizes of the PCR product amplified and generated consisted of 1 cycle at 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 2 min, and finally 1 cycle at 72°C for 5 min. The 905-bp sequences of the *gyrA* genes of other clinical *Arcobacter* isolates (*A. butzleri* strains 235-2004, 1285-2003, 1188-2003, 1477-2003, 1172-2003, and 1426-2003 and *A. cryaerophilus* strains 322H-2004, 622H-2004, 492-2004, PC367, and PC249) were amplified using this PCR assay and sequenced on both strands with PCR primers using an Applied Biosystems 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA) with a fluorescence BigDye Terminator V1.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions (Ramees et al., 2014).

Occurrence And Prevalence Of *Arcobacter* In Dogs And Cats

In pets and other companion animals, various studies around the globe have been carried out and they indicated different carriage rates of *Arcobacter* (Abdelbaqi et al., 2007; Gonzalez and Ferrus, 2011; Gude et al., 2005; Lehner et al., 2005). This could be attributed to the sensitivity of the different isolation and confirmation methods used. The carriage in pets may contribute to their transmission within the environment (Collado and Figueras, 2011). Fera et al. (2009) reported 78% of cats were positive for *Arcobacter* in a study conducted in Southern Italy and Takahara et al. (2008) reported 4% of dogs sampled were positive for *A. butzleri* and *A. cryaerophilus*; on the contrary, Houf et al. (2008) showed cats did not harbour the organism but isolated them from dogs (2.6%). However, none of the samples collected from dogs in Turkey were positive for *Arcobacter* (Aydin et al., 2007). *Arcobacter butzleri* was isolated from the saliva of cats and dogs that had oral/dental conditions conducted in a study in Denmark and Malaysia (Goni et al., 2016). *Arcobacter butzleri* and *A. cryaerophilus* were the species isolated from dogs and cats in studies conducted in Japan, Italy and Belgium (Takahara et al., 2008; Tenkate and Stafford, 2001). In Japan and Australia, isolates sampled showed the occurrence rates 7% and 2.2% for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* respectively (Kabeya et al., 2004; Rivas et al., 2004). *Arcobacter butzleri* is the most frequent specie followed by *A. cryaerophilus*. *Arcobacter skirrowii* has a very low prevalence and were seldom or not isolated at all (Houf et al., 2003; Houf et al., 2008; Öngör et al., 2004) like shown in various studies in Table 2.

Public Health Significance

Several studies have shown an increasing trend in the occurrence of *Arcobacter* in dogs, cats and humans, hence raising public health concern. Similarly, *Arcobacter* have been isolated from various sources across the globe ranging from poultry, dogs, cats, beef, milk and the environment that are considered as potential sources of human infection. They are considered as emerging pathogens and detected from enteritic and septicemic patients blood of uremic patients with hematogenous pneumonia and a traffic accident victim

Table 2: Prevalence rate of *Arcobacter* species across the globe from different studies

Author	Prevalence	Species isolated	Country
Houf et al. (2008)	Dogs 2.6% (7/267), Cats 0% (0/61)	A. cryaerophilus and A. butzleri	Belgium
Fera et al. (2009)	Cats 78.8% (67/85)	A. cryaerophilus and A. butzleri	Italy
Pejchalova et al. (2015)	Dogs 3.7% (4/108), Cats 1.4% (1/70)	A. cryaerophilus	Czech Republic
Goni et al. (2016)	Dogs 54.4% (55/101), Cats 39.5% (34/86)	A. butzleri	Malaysia
Aydin et al. (2007)	Dogs 0% (0/65)		Turkey
Patyal et al. (2011)	Dogs 0% (0/75)		India
Takahara et al. (2008)	Dogs 4% (6/157)	A. cryaerophilus and A. butzleri	Japan

(Arguello et al., 2015; Prouzet-Mauleon et al., 2006; Engberg et al., 2000). Dogs and cars are significant reservoir for human infection with *Arcobacter* species and has been associated with infection of *A.butzleri* diarrhoea in man due to their close contact with pet owners (Douidah et al., 2014; Goni et al., 2016). The prevalence of *Arcobacter* in human infections has probably previously been underestimated because of inappropriate detection and typing methods from stool samples (Bhatti et al., 2016). *Arcobacter* species should be considered and tested for in cases of diarrheal disease such as traveller's diarrhoea in humans with clinical signs of persistent watery diarrhea which is seldom bloody with abdominal pain, nausea and vomiting or sometimes fever (Whiteduck-Léveillée et al., 2015; Tauxe, 1997). *Arcobacter* have been known from various studies to cause reproductive problems though infection can be symptomatic or asymptomatic in dogs and cats. It often results in infertility, chronic discharge during estrus, chronic still-births problem and enteric problems (Ho et al., 2006; Vandenberg et al., 2004). *Arcobacter butzleri* produces watery diarrhea by inducing epithelial barrier dysfunction making changes in tight junction proteins and induction of epithelial apoptosis by which leak flux type of diarrhea occurs (Bucker et al., 2009).

Antibiotic Resistance

Arcobacter like other emerging zoonotic disease-causing organisms are reported to be increasingly resistant to antibiotics mainly due to their widespread overuse in animals. Antibiotics are often regarded as one of the wonders of the 20th century; however the wonders raised by the issue of antibiotic resistance cannot be overemphasized. The potential development of resistance by these microbes have compromised the benefits of antimicrobial agents (Davies and Davies, 2010). Resistant *Arcobacter* species can be transferred to humans through direct ingestion of contaminated food or through contact with animals. The World Health Organization (WHO) has suggested a halt in use of antibiotic as growth promoters that belong to an antimicrobial class used in human medicine. The continued usage of excess antibiotics in animals makes animals more susceptible to acquisition of the resistant strains of the organism (Angulo et al., 2004).

The public health consequences of antibiotic use in animals can be evaluated more importantly when consideration of each pathogen-antibiotic situation (Phillips, 2001). Multidrug resistance has been reported in both *Arcobacter* species from various studies (Ho et al., 2006; Son et al., 2007). The occurrence of *Arcobacter* varies according to animal species and geographical location. Son et al. (2007) in a study in the United States showed the prevalence of antibiotic resistant *Arcobacter* to be 93.7% to one or more antibiotics. Similarly, 71.8% are resistant to two or more antibiotics. The resistance displayed by *A. butzleri* isolates to clindamycin (90%), azithromycin (81.4%) and nalidixic acid (23.6%). *Arcobacter butzleri* isolates were found to be highly resistant to β-lactams, antibiotics based on a study conducted in Poland, on the other hand, only one isolate of *A. cryaerophilus* was susceptible to erythromycin. Tetracycline and aminoglycosides showed the highest susceptibility against *A. butzleri* and *A. cryaerophilus* (Zacharow et al., 2015). The variation in the occurrence of antibiotic susceptibility among *Arcobacter* species suggests that appropriate antibiotics should be selected for the treatment of infections and when developing isolation media for the wide range of *Arcobacter* species (Ünver et al., 2013).

Houf et al. (2001) conducted a study on antimicrobial susceptibility of *Arcobacter* and designed with a protocol for the isolation of *Arcobacter* that is frequently used. This method involves selective isolation supplemented with antibiotic (cefoperazone, amphotericin B, 5 fluorouracil, novobiocin and trimethoprim) in the enrichment and plating medium. This method is very popular because it is the only method that has been recognised for isolation of *Arcobacter* from faecal specimens (On, 1996). So far, there are paucity of studies on the resistance mechanism of *Arcobacter* species, however, few studies revealed the only resistance mechanisms chromosomal nature as the main mechanism of antibiotic resistance and no genes coding to antibiotic resistance were identified in plasmids (Harmon and Wesley, 1997; Ramees et al., 2014).

In some studies conducted in cattle farms, it showed a 3.7% prevalence rate of antibiotic resistant *Arcobacter.* The *A. butzleri* isolates were sensitive to ampicillin, erythromycin, tetracycline, cefotaxime, gentamicin, ciprofloxacin,

nalidixic acid, enrofloxacin and chloramphenicol. However they were found to be highly resistant to ampicillin (55.6%) followed by cefotaxime (33.4%) and ciprofloxacin (33.4%) with 20% of all the isolates showing multidrug resistance (Shah et al., 2013). Variation in the resistance patterns could be due to the irrational and frequent use of drugs in animals for therapy and/or prophylaxis, however it could also be due to the unavailability of standardization for *Arcobacter* antibiotic susceptibility tests and lack of well recognized breakpoints. The food contamination with resistant bacteria may also lead to a transfer of antibiotic resistance factors to other pathogenic bacteria leading to failed treatments of chronic infections (Morita et al., 2004).

CONCLUSION

In conclusion, detection and public health significance of *Arcobacter* in dogs and cats is becoming a major concern across the globe. Occurrence of *Arcobacter* species may be underestimated, either because a possible previous misidentification or due to the paucity of studies concerning the assessment of new species prevalence. However, there detection in pets and stray animals are important because the presence of these emerging foodborne pathogens pose a risk to the human population and constitutes a public health concern. It is evident that they are associated with diseases in humans and animals. The shedding of these organisms by stray dogs and cats may be a source of contamination of the environment. Also, the irrational and habitual used of antibiotics is a key factor in the increase and spread of antibiotic resistance.

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CONFLICT OF INTEREST

There is no conflict of interest in this review to declare.

AUTHORS' CONTRIBUTION

All the authors contributed equally for plan of review, article collection and manuscript writing.

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