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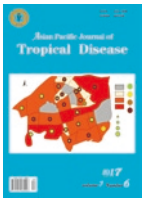

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Campylobacter in the environment: A major threat to public health

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ABSTRACT

Epidemiological data suggest that *Campylobacter* remains a worldwide leading cause of gastrointestinal infections. Improperly prepared meat products, unpasteurized milk as well as non chlorinated drinking water were shown to be the main sources of campylobacteriosis. The *Campylobacter* survival mechanism in various environments facilitated the transmission of *Campylobacter*-associated infections; however the exact mode of transmission remains to be elucidated. This review aims to summarize recent insights on the incidence and survival of *Campylobacter* in the environment. Besides, methods of detection and risk assessment for public health safety are also addressed.

1. Introduction

The genus *Campylobacter* was first described in 1906 by John McFadyean and Stewart Stockman from the uterine mucous of sheeps. Early classification of *Campylobacter* was considered as a member of the genus *Vibrio* in 1919 with a typical species known as *Vibrio fetus*. Later, the isolation of *Vibrio jejuni* was reported from the jejunum of calves with diarrhea in 1972, followed by the isolation of *Vibrio coli* from pigs in swine dysentery in 1944[1,2]. Due to their fundamental differences from other *Vibrio* spp. revealed by various taxonomic studies, *Vibrio fetus*, *Vibrio jejuni* and *Vibrio coli* were reclassified, and that led to proposal of a new genus, *Campylobacter*[3]. Although *Campylobacter* spp. have been known as an important veterinary pathogens for many years, their role as a cause of enteric infection in humans was not recognized until the mid 1970s, when Skirrow[4] managed to isolate the organism, using solid cultivation media, from fecal samples of diarrhea patients. Since that time, *Campylobacter* spp. have been recognized as the leading cause of human gastrointestinal infections in both industrialized and developing countries[3,5].

Gastroenteritis or campylobacteriosis is characterized by watery diarrhea that sometimes contains blood, usually accompanied by abdominal cramping[3]. The infection is characterized by a spectrum of clinical manifestations, from complete absence of symptoms to full acute colitis. Diarrhea and cramping are the most common symptoms. After ingestion of *Campylobacter* cells, it usually requires between 24 to 72 h for incubation period; then the first symptoms that can be observed is fever at 40 °C; lasting for up to 2 days, followed by other symptoms that may include: nausea, severe abdominal cramp, malaise and vomiting. *Campylobacter*iosis is a self-limiting infection that lasts 2–7 days[3].

The infection required to cause campylobacteriosis is thought

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to be low, around of less than 500 cells. Individuals with campylobacteriosis may remain carriers for *Campylobacter* spp. for up to 2 weeks, as they shed around 10^6 to 10^8 campylobacters in one gram of their feces[3,6]. There might be further complications that follow campylobacteriosis, these may include meningitis, inflammation of gall bladder, urinary tract infection, bacterimia, urethritis and arthiritis (Reiter's syndrome) and reverse paralysis (Guillian-Barre' syndrome); fatalities due to campylobacteriosis are very rare[3,6].

Thermotolerant campylobacters, *i.e. Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*) and to some extent *Campylobacter lari* (*C. lari*) are the species most encountered in waterborne and foodborne infections[5]. It is believed that the majority of *Campylobacter*-associated infections are sporadic cases of food poisoning, nevertheless, contaminated water supplies are probably the vehicle for *Campylobacter*-associated outbreaks. Despite the fact that *Campylobacter* spp. are widespread in the environment, the epidemiology of many of cases of campylobacteriosis remains unclear[5]. This article aims to shed some light on the occurrence and survival of campylobacters in various environments, and its detection in environmental samples. Further, the risk assessment for public health is briefly discussed.

2. Epidemiological aspects

Epidemiological data of campylobacteriosis have revealed an increasing important role of *Campylobacter* infections in public health. Despite global efforts that successes, in part to control the transmission of other enteric pathogens, the prevalence of *Campylobacter* infections nonetheless continued to increase across the world[3].

Free living wild birds and migratory waterfowl appear to be an important environmental reservoir of *Campylobacter* species. The intestinal carriage of campylobacters in wild birds (*e.g.* crows, ducks, geese, pigeons, magpies, house sparrows, and various other species), have been frequently isolated from the feces of apparently healthy birds without obvious clinical manifestations associated with *Campylobacter* infections[5,7-11]. This observation suggests that *Campylobacter* species inhabiting the guts of wild birds are regarded as commensals; an observation that might be related to the body temperature of the birds (42 ℃) being the optimal growth temperature for thermotolerant campylobacters[5,7].

Campylobacter species inhabit the intestinal tract of warm-blooded birds and animals, therefore they are exogenous to aquatic environments, and so far, its capability to grow in water is unproven, thus, the apparent wide distribution of campylobacters in aquatic environments is believed to be due to recent fecal contamination from sewage effluents and/or agricultural runoff or direct fecal contamination from wild birds and animals[5]. *Campylobacter* species were readily detected from fecally contaminated drinking water[12], rivers[13, 14], lakes[15], ponds[16], drainage channels[14], ground water[17,18], marine waters[19], rain-related and agricultural runoff[16], additionally, sewage effluents contain abundant concentrations of *Campylobacter* species[20,21]. Drinking untreated water plays an important role in *Campylobacter* epidemiology as it remains one of the most significant sources of campylobacteriosis worldwide[12].

The incidence of campylobacteriosis in the community is also related to the consumption of contaminated food and milk. The presence of the *Campylobacter* species in the intestinal tract of chickens is well documented, where campylobacters were isolated from fecal droppings, cloacal swabs and cecal contents of chicken[22-24]. Poultry are recognized as a major environmental reservoir of *Campylobacter* sp. despite the fact that the bacterium is not one of the normal components of intestinal flora of the chickens[25]. Thus, colonization of chicken by *Campylobacter* is most likely due contamination of water supply of the broiler or the broiler environment[22,26,27]. Chicken carcasses obtained from supermarkets and slaughterhouses were found to be heavily contaminated with campylobacters[25-27]. The source of carcass' contamination is the birds themselves, yet the processing and packing could cause cross-contamination[25,27]. Epidemiological data showed significant evidence indicating that undercooked chicken meat and/or handling of chicken is a major source of *Campylobacter* enteritis in the communities[25,27]. Similarly, several investigations reported the presence of thermotolerant campylobacters in the intestinal tract of cattle, sheep and pigs. Livestock can act as asymptomatic carriers of campylobacters and animal food products can be contaminated by this pathogen during slaughter and carcass dressing. Therefore the consumption of contaminated undercooked meat and drinking contaminated pasteurized or raw milk is predominantly a potential source of human campylobacteriosis[28].

The consumption of unpasteurized milk represents a common source of human campylobacteriosis. Outbreaks of *Campylobacter* infection associated with drinking unpasteurized milk are well documented[29,30]. Similarly, small outbreaks of campylobacteriosis within farming families associated with drinking raw milk originating from their farm are frequently reported[31,32]. *C. jejuni* is the most common species causing milk-borne outbreaks. Milk may be contaminated by *Campylobacter* with fecal matter from cows during milking process, poorly sanitized milking equipments, contamination during repairs of milking machines and secretion of the organism by cows with mastitis[29].

Domestic pets, such as dogs and cats that live in close proximity to humans, have been responsible for a wide range of bacterial and parasitic zoonoses. These animals have also been found to carry *Campylobacter* in their intestinal tract, both healthy and diseased individuals[33,34]. These findings suggested that cats and dogs are a potential reservoir for human-pathogenic *Campylobacter* spp. and may constitute a potential risk factor for human campylobacteriosis, particularly in children[11].

3. Environmental sources of *Campylobacter*

Despite being considered as 'fragile and fastidious' organism that is unable to live outside its host, campylobacters are widespread in the environment. The exact role that various environments play in the epidemiology of campylobacters infection remains to be elucidated.

3.1. Drinking water

Several waterborne outbreaks of campylobacteriosis associated

with drinking were reported in the past twenty years, often affecting thousands of individuals, however, many outbreaks remain unreported in many countries worldwide due to lack of specific notification system and/or testing[12]. The first outbreak of drinking water-associated campylobacteriosis was reported in Bennington, Vermont, USA in 1978, where about 3 000 individuals had enteritis after drinking unboiled water from the town water system, and the investigation into that outbreak showed that the source of contamination was a main unflitrated water source[35]. Drinking water-associated *Campylobacter* outbreaks have always been linked to either disinfected drinking water or cross-connections and water treatment breaks as a result of sewage contamination or heavy rainfall[6,12]. *C. jejuni* was the common species implicated in waterborne outbreaks worldwide, and other species such as *C. coli* and *C. lari* have very rarely been encountered[36-38]. Despite the large numbers of waterborne campylobacteriosis outbreaks, the organisms causing the outbreaks have rarely been isolated from drinking water[6,12]. This is perhaps due to sporadic occurrence or the presence of viable but nonculturable (VBNC) *Campylobacter*[6].

3.2. Surface water

Aquatic environments are believed to be an important environmental reservoir of campylobacters. The presence of *Campylobacter* spp. in environmental surface water is strongly related to waterfowl and wild birds, sewage treatment plants, and rain-related runoff[5]. In France, the incidence of *Campylobacter* spp. in five different rivers in the neighborhoods of Saint-Brieuc, Brittany was investigated over a 12 months period and was found that 50% of the samples were positive for *Campylobacter* species. *C. jejuni* was the predominant species in all five rivers, and its sources were linked to poultry (runoff from poultry farms) and human origins (sewage discharge) based on PFGE profiles, whereas *C. coli* was sharing profiles with isolates from pigs (runoff from swine farms) and *C. lari* were related to wild birds[39]. In amenity village ponds at South Dalton, Little Weighton and Brantingham in the UK, *Campylobacter* spp. were linked to the presence of free living wild ducks and geese at South Dalton and rain-related runoff at Little Weighton and Brantingham, where *C. jejuni* was found to originate from direct deposit of birds feces and *C. coli* was linked to rain-related runoff, that could carry the bacteria from the feces of dogs and horses on the road or from nearby swine farms[16]. In Luxemburg wild birds were found to be the predominant contributor to *Campylobacter* contamination in streams/canals (63%), ponds/lakes (85.2%) and rivers (85%), whereas in the Netherlands, poultry was the main source of campylobacters in streams/canals, and wild birds were contributing to the contamination of rivers/ponds and lakes. The diversity of *Campylobacter* species in both countries showed that *C. jejuni* in surface water was always genetically related to wild birds and poultry, while *C. coli* was related to agricultural practices (runoff from agricultural land and farms)[40]. In marine (Morecambe Bay) and fresh (Rive Lune) bathing waters, *Campylobacter* spp. originate from a diversity of sources, primarily, sewage effluents, runoff from agricultural land and dense populations of wild birds. *C. lari* and urease-positive thermotolerant campylobacters (UPTC) were the only species detected at Morecambe Bay, while in River Lune, *C. jejuni*, *C. coli*, *C. lari* and UPTC were detected. The sources of *C. lari* in the former site was found to be dense populations of gulls, knots and oystercatchers, whereas sewage effluents, runoff from agricultural land and dense populations of ducks were the source of *C. jejuni*, *C. coli*, *C. lari* and UPTC at the latter site[19,41]. Contamination of the River Leck (Netherlands) with *Campylobacter* sp. was in part, attributed to the gastroenteritis infection in group of athletes who were competing in an Olympic triathlon, probably due to accidental ingestion of contaminated river water[42].

3.3. Sewage effluents and sludge

Campylobacter species are ubiquitous in sewage, human and animal wastes from farms, abattoirs and animal processing plants are major sources of *Campylobacter*'s contamination in the environment[20,21,43-45]. Studies from England[43] showed that the numbers of campylobacters in sewage were related to the incidence of campylobacteriosis in the community and the presence of animal effluents from abattoirs and poultry processing plants. The presence of *Campylobacter* in sewage was found to exhibit an identical seasonal pattern to incidence of campylobacteriosis in the community; with a large peak in May and June and minor one in September and October[43,46]. This observation may suggest that the increase of campylobacteriosis in the community are related to the increase of campylobacters amounts in the environment, which in turn correlated to the changes in the numbers of *Campylobacter* spp. within poultry, livestock and wild birds[47].

Treatment of sewage by primary settlement showed slight reduction of the numbers of campylobacters by 78%[48]. Considerable reduction of *Campylobacter* in sewage can be achieved by secondary treatment (*i.e.* trickle filtration, activated sludge and oxidation ponds) where the numbers of campylobacters could be reduced by 88% to over 95%[46,49]. Complete elimination of campylobacters from sewage effluents can be achieved by tertiary treatment and chlorination[50]. Fresh sewage sludge contains high numbers of campylobacters, however, sludge digestion could eliminate the numbers to zero[47]. The application of improperly treated sewage effluents and sludge to agricultural activities may act as source of campylobacters in the community[43,50].

3.4. Wild birds

The natural coexistence of *Campylobacter* species in the intestinal tract of various taxa of wild birds and migratory waterfowl makes these birds an important source of *Campylobacter* contamination in the environment as well as disseminating campylobacters in various locations via long distance migrations[5,7]. In addition to their role in contributing abundant numbers of campylobacters to aquatic environments (*e.g.* rivers, lakes, marine waters), wild birds were implicated directly to waterborne and milk-borne *Campylobacter* outbreaks. In Greenville, Florida, USA an epidemic waterborne campylobacteriosis (*C. jejuni*) associated with a community water supply affecting 865 individuals. Epidemiologic, laboratory and environmental investigation suggests that wild birds (grackles; house sparrow; cardinal; ground dove) perching and defecating near the open-top water tank of the community water supply was

the only possible source of *Campylobacter* contamination[51]. In the United Kingdom, a series of milk-borne campylobacteriosis (*C. jejuni*) were strongly associated with wild birds (Jackdaws; magpies) contaminating milk bottles by pecking the bottle tops, on the doorsteps[52-55]. Wild birds may also be in part, responsible for the contamination of chicken broiler house with campylobacters. *Campylobacter* spp. are frequently isolated from wild-bird feces around broiler houses, and studies with molecular epidemiological tools have reported that the strains isolated from such samples can, on occasion, subsequently be recovered from the cecae of broilers in those houses[56].

4. Detection and typing of camplyobacters in environmental samples

Detection and typing of *Campylobacter* from environmental sources requires both cultural based, and molecular, PCR, based methods. Initial collection and processing of samples may differ depending on the density of the bacterium, nature of samples and the laboratory techniques employed. A brief description is given below.

4.1. General aspects

The type of culture media and incubation temperature are determinant factors in the detection of campylobacters in environmental samples (feces; water; food). Various comparative studies evaluated the suitability of different enrichment and plating culture media for the recovery of *Campylobacter* spp. from environmental samples suggested that Preston enrichment broth, Preston agar and modified charcoal-cefoperazone-deoxycholate (mCCDA) were found to be superior as they gave fewer problems with contaminant microflora[57,58]. Various *Campylobacter* selective agars (*e.g.* Butzler, Skirrow, Campy BAP) were usually produced plates with contaminant flora (*e.g.* coliforms, *Pseudomonas* spp., yeast) that could attribute to poor or no recovery of target organism[57,59].

Enrichment step is used to resuscitate injured cells; therefore, the enrichment of environmental samples in basal or selective broth significantly increases the recovery of damaged *Campylobacter* cells. An incubation regime that involved a 4-h incubation in selective broth culture (*e.g.* Preston broth: nutrient broth containing 5% lysed horse blood, 0.02% sodium metabisulphite, 0.02% sodium pyruvate, and 0.05% ferrous sulfate) at 37 ˚C, prior to incubation for 44 h at 42 ˚C improved the recovery of injured *Campylobacter* cells in water[60], bird feces[16], milk and dairy product[61], probably by allowing repair of sublethally injured cells prior to their exposure to higher temperature[62,63]. In general the use of Preston broth for selective enrichment and mCCDA for selective plating combining together has been recommended for the detection of *Campylobacter* spp. in drinking and environmental water samples where *Campylobacter* spp. usually occurs in relatively low numbers or sublethally injured[5].

The optimal incubation temperature and duration for the detection of campylobacters on selective agar is reported as 42 ˚C for 48 h[58]. Observing the plates after 24 h probably increases the possibilities of false-negative results, whereas scoring the plates after 72 h increases the overgrowth by contaminant microflora (*e.g. Pseudomonas* spp., *Proteus* sp.)[58].

4.2. Detection in feces

Detection of campylobacters in the feces of diarrhea patients can be achieved by direct plating onto *Campylobacter* selective agar (*e.g.* Preston agar; Modified charcoal, cefoperazone and deoxycholate agar [mCCDA]) plates[64,65], as high number of target organism are present. Similarly, direct plating onto selective media has been frequently used for the detection of campylobacters in avian/animal fecal samples and/or cecal contents[9,11,66]. Although direct plating of fecal materials on *Campylobacter* selective agar plates has been successfully employed in detecting the organism in human and birds/animal fecal samples, it might guarantee a high recovery rates of campylobacters particularly from avian and animal origin or might result in failure to detect the organism in feces[67,68], therefore, incubating fecal samples in selective enrichment broth prior to selective plating potentially increase the detection of campylobacters[5]. Indeed, the incubation of avian/animal fecal samples in enrichment broth prior to selective plating was successfully employed in detecting *Campylobacter* species[10,16,69-71].

The application of molecular detection methods has been successfully employed to the determination of campylobacters in the feces of diarrhea patients[72,73] and in avian/animal fecal samples[74]. Detection of *Campylobacter* spp. in feces by direct PCR (direct detection of target DNA from samples)[75] or by PCR after a selective enrichment step (detection of target DNA from enrichment broth culture incubated at 37 ˚C for 4 h followed by 48 h at 42 ˚C), has been applied successfully to human, avian and animal feces[76-78]. Determination of campylobacters in feces by PCR provides more accurate account of the incidence of the organism, furthermore, PCR potentially reduces the time for detection and eliminates the need for conventional confirmatory methods[5,73].

4.3. Detection in water and wastewater

The detection of campylobacters in water samples usually involves their concentration, from large volumes of water, onto membrane filters. The filters usually incubate in a selective enrichment broth at 37 ˚C followed by incubation at 42 ˚C, and streaking of broth cultures onto selective agar plates that are subjected to further incubation of the plates at 42 ˚C for 48 h[5]. Such approach has been employed for the detection of *Campylobacter* spp. in drinking water[79], rivers[80], ponds[60], rain-related runoff water[16], marine waters[81] and sewage effluents[82]. The filtration of larger volumes of water samples may be appropriate to increase the number of *Campylobacter* cells concentrated on the filters[5]. Thus, to increase the number of campylobacters detected in drinking water, it was recommended to filter up to 10 L, the filtrations of 1 000 mL sample volumes, however, are too small for the routine detection of campylobacters in drinking water[79]. Similarly, it was suggested that the filtration of large volumes (*e.g.* 1 000 to 4 000 mL) may be appropriate for the determination of campylobacters in environmental waters[80,83]. With groundwater, the filtration of a 10 mL sample failed to recover

campylobacters[84], while, successful detection of *Campylobacter* was achieved with the filtration of 100 to 500 mL of groundwater[17]. However, the filtration of large volumes (1 000 mL) of turbid environmental waters or heavily contaminated sewage effluents can lead to false-negative results. This is because of the growth of high levels of background heterotrophic bacteria and coliforms during the enrichment stage prevents the growth of campylobacters to detectable levels, possible because of competition for nutrients, thus, with turbid surface water, the filtration of 10 to 100 mL proved appropriate for the determination of campylobacters[60].

The molecular techniques based detection of *Campylobacter* spp. in water and wastewater were successfully achieved employing various PCR protocols, using diverse primers[18,85-90]. Most of these protocols aimed at the detection of the presence or absence of campylobacters; whereas other protocols[91] used real-time PCR for obtaining quantitative results. The application of direct PCR assay, without the need for enrichment culture, to detect naturally-occurring campylobacters in contaminated drinking water and swimming pool water has been reported[92,93]. Thus, although direct detection of *Campylobacter* spp. by PCR in drinking water may be possible, the direct application of PCR to turbid environmental samples may encounter few problems and probably give false negative results[88]. As in turbid environmental waters, *Campylobacter* spp. are usually present in relatively low numbers against an abundant background microflora and potential PCR inhibiting substances, which is not the case in contaminated drinking water or swimming pool water.

The combination of PCR assay after selective enrichment step may increase the effectiveness and sensitivity of PCR detection of campylobacters in environmental waters, prpobably by increasing the number of target cells. This was noted with deliberately seeded environmental waters with known concentrations of *Campylobacter* cells[18,86], as well as with naturally-occurring campylobacters in environmental waters and sewage effluents[13,18,82,86,88-90]. Thus, with PCR assay performed on spiked enrichment cultures the minimum detection limits of 3, 13 and 30 cells per 100 mL were obtained for river and waste water[86] and estuarine water[94] respectively, while in naturally contaminated turbid pond water, 400 cells per 100 mL were required for direct detection by PCR after selective enrichment step[88].

The application of direct PCR for the detection of campylobacters in environmental samples, without enrichment step, may lead to the detection of naked DNA fragments or DNA amplified from dead *Campylobacter* cells[5]. The presence of dead *Campylobacter* cells in environmental waters may insignificantly pose any threat to public health[88]. Therefore, the detection of viable *Campylobacter* cells in environmental waters is the issue of concern to public health authorities. In this respect, the application of a PCR assay after selective enrichment step, may significantly encourage the detection of viable cells only. PCR assay after selective enrichment is being considered as a standard method for the detection of presence or absence of campylobacters in environmental samples[95,96]. The use of fluorescence in situ hybridization (FISH) can be helpful in distinguishing whole cells from DNA fragments in detecting campylobacters in environmental samples, by means of labeling whole cells using a fluorescent *Campylobacter*-specific oligonucleotide probe and then observing these cells under an epifluorescence microscope[97].

4.4. Detection in food and milk

Various media and methods have been proposed for the detection of *Campylobacter* spp. in food and dairy products[98,99]. The recommended incubation temperature throughout the isolation process is 42 ˚C. Selective enrichment of samples in broth culture containing cefoperazone and amphotricine B, followed by selective plating on mCCDA is the best choice. The use of multiplex PCR (mPCR) is rapid, simple method to identify *Campylobacter* spp. to species level[100].

4.5. Typing of campylobacters

The typing of confirmed *Campylobacter* isolates in an important tool for obtaining epidemiological information related to the following aspects: (i) tracking the route of transmission to humans; (ii) monitoring the geographic and temporal distribution of specific strains; (iii) developing control strategies[101]. Various methods are used for typing *Campylobacter* isolates; these methods include serotyping, phenotyping, phage typing, and molecular-genomic typing protocols.

Serotyping methods, detect the agglutination of specific antigens with antisera[102]. These methods are usually used in clinical settings, however they have the problem of ‘untypable strains’ strains of confirmed *Campylobacter* isolates, from human and environmental are frequently encountered[81,103,104]. A combination of phage typing[105] and serotyping can help with this problem[101]. Phenotyping methods usually take the forms of biochemical tests such as hippurate hydrolysis; H_2S production, these methods may prove valuable for differentiating between *Campylobacter* spp, in particular *C. jejuni* and *C. coli*[106], however, one of the main disadvantages of phenotyping methods is their limited ability to discriminate between many species of the family Campylobacteriaceae[107].

DNA-based typing methods are more sensitive and accurate than conventional techniques. Genomic-based typing techniques include: digestion of bacterial DNA using restriction enzymes prior to pulsed-field gel electrophoresis (PFGE)[108]; random amplification of polymorphic DNA (RAPD)[109]; amplified fragment length polymorphism fingerprinting (AFLP)[110]; polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[111]; flagellin typing (fla-RFLP)[112]; direct nucleotide sequencing (with or without prior PCR)[16,113]. Multilocus sequence typing (MLST) is widely used now to differentiate between *C. jejuni* and *C. coli* and has been recognized as an accurate typing tool for the differentiation of *Campylobacter* spp. due to its high sensitivity[112,114-116].

4.6. Survival strategies/mechanisms of campylobacters in the environment

Campylobacter spp. are exogenous to all environments (*e.g.* water, soil, food) and there is no available evidence so far of its ability to grow in any environment other than in their warm-blooded hosts and laboratory culture media. However, campylobacters are capable

of surviving for prolonged periods in various environments under certain conditions.

In aquatic environments (*e.g*. drinking water, environmental fresh and marine waters), campylobacters were found to survive for up to 4 months at lower temperature (4–5 ℃)[117-122]. Temperature is an important determinant factor for the survival of *Campylobacter* spp. in water[123], cold temperatures favors for the survival of campylobacters in water, when temperature increases (16 ℃ and higher), *Campylobacter* populations in water decline rapidly. This is perhaps due to an increase in metabolic activities and rapid utilization of all nutrients available at the expense of other functions including culturability[117].

Survival of *Campylobacter* species in aquatic environments was also found to be affected by light (UV radiation). A rapid decrease of campylobacters pure cultures and natural population numbers by more than one order of magnitude was observed after less than 10 min of exposure to stimulated sunlight in fresh and marine water microcosms[124]. Although light affects changes in the uptake of nutrients and inhibit the active transport and biosynthesis in *Escherichia coli* (*E. coli*)[125], the effects of light on campylobacters remains to be fully elucidated[5].

Other factors that play an important role in the survival of *Campylobacter* spp. in the environment include predation and nutrient availability[126]. *Campylobacter* spp. were found to survive better in lake water that had been filtered through 0.2 mm cellulose nitrate filters than in unfiltered lake water, probably due to the removal of zooplankton predators and reduced competition for nutrients; for example, *C. jejnui* was found to survive for 10 days at 4 ℃ in filtered environmental water[118]. Furthermore, *C. jejuni* was able to survive, in culturable forms for more than 20 days at 4 ℃ in non-autoclaved, 0.2 μm-filtered, lake water, however when the same water samples were autoclaved, *C. jejuni* was able to survive less than 10 days, suggesting that either autoclaving had destroyed some key heat-liable nutrients required by campylobacters, or possibly produced toxic by-products that may have affected the cells[127].

Biofilm formation is common survival strategy for *Campylobacter* spp. in harsh environments[123], *C. jejuni* can form biofilm in aquatic environments and on various surfaces in water distribution systems and food processing industries[128]. Aerobic conditions and low concentrations of nutrients can promote biofilm formation by campylobacters so they can survive within polymicrobial biofilm[129-131]. It was hypothesized that mixed microbiota within biofilm community may act to improve the survival of campylobacters in the environment (outside the host) and may also protect the pathogen against the intestinal immune system, possibly by gaining micronutrients from other organisms or hiding in and acquiring nutrients from amoeba[132].

Different *Campylobacter* species may exhibit different survivability rates in aquatic environments; this is very relevant to public health concerns because *C. jejuni*, which is ubiquitous in diverse environment, is the major cause of human campylobacteriosis. Korhonen and Martikainen[118] found that *C. jejuni* survived longer than *C. coli* in both unfiltered and 0.2 μm-filtered lake water microcosms incubated both at 4 ℃ and 20 ℃. Similarly, in a study conducted by Thomas *et al*.[120], it was found that C. *jejuni* survived better than *C. coli* and *C. lari* under various conditions; for example; in autoclaved de-ionised water; autoclaved environmental water; autoclaved river environmental with sediments; incubated at 5 ℃, 15 ℃, 25 ℃ and 37 ℃. Conversely, Obiri-Danso *et al*.[124] tested the survival of *C. jejuni* and *C. coli* in artificial sea water, and reported that *C. jejuni* and *C. coli* had similar survival times, and that both of these species survived less well than *C. lari* and UPTC. These conflicting results from different studies probably, due to variations between strains used in every study, variations of experimental systems, and growth history of the organisms[133].

Survival of campylobacters in water may also be strain-dependant (*i.e*. depends on the origin of the strain). In a study conducted by Cools *et al*.[134], it was found that the majority of *C. jejuni* strains originated from natural waters and clinical sources were unable to survive more than 29 days in drinking water microcosms stored at 4 ℃. Whereas poultry derived strains were able to survive for longer periods of time (30–52 days) in the same incubation conditions, this feature could contribute to the persistence and the spread of *C. jejuni* in the environment. Trigui *et al*.[121] investigated the survival of various isolates of *C. jejuni* originated from chicken ceca in fresh water microcosms and observed great variability in the survivability of different strains. This variation is probably explained by the variation of genetic content between these strains.

Various reports have explored the survival of *Campylobacter* spp. in dairy products; meat and food processing surfaces. Hong *et al*.[135] studied the survival of *C. jejuni* on various processed meat products (*e.g.* dry-cured ham; round ham with or without sodium nitrite) and noted that *C. jejuni* populations declined rapidly below detection limits on dry-cured ham within 40 days (expiration date) stored at 4 ℃ and 10 ℃, while at 36 ℃, *C. jejuni* declined to 1 log CFU/g within 24 h. This might be explained by characteristics of dry-cured ham such as low water activity, low pH and high salinity. Similar observation was also noted with other types of processed meat and that led to the conclusion that regardless of the type of processed meat, the public health risk posed by these types of meat is relatively low. *C. jejuni* of animal origins was able to survive on beef trimmings for up to 112 days during freezing and frozen storage (–18 ℃)[136]. On chicken skin, the natural populations of *C. jejuni* was found to survive for 84 days at –20 ℃, while on minced meat treated with 1.5% NaCl, *C. jejuni* was able to survive for 14 days at 4 ℃[137]. In moderately salted cheese *C. jejuni* survived for 7–14 days while in yogurt it was found that *C. jejuni* was detectable for 2–3 days, which suggest that the low pH of yogurt was the determinant factor of low survival ability of *C. jujuni*[138]. The ability of campylobacters to survive in different food matrices under storage conditions nesseciates the development of new strategies to control this pathogen in the food chain industry.

Various phenotypic and genotypic methods were used to elucidate the mechanisms influencing the survival and persistence of *Campylobacter* spp. in the environment (water, food, feces, soil, surfaces) and the role of this survival may play in the transmission of *Campylobacter* to human hosts, however, the reported variation between different strains of *C. jejuni* (and variation between different *Campylobacter* spp.) makes it difficult to draw species wide conclusions based single strain studies. Therefore, the real challenge now is to link between all the data generated from both phenotypic and genotypic survival studies in order to fully understand the role

of environmental survival in the transmission of *Campylobacter* spp. [123].

5. The VBNC state

The VBNC state refers to the ability of bacterial cells to remain viable by retaining basal metabolic activities, but unable to grow in laboratory culture media. In enteric bacterial species, this state is assumed to be a survival strategy when these cells are released into the environment and suffer prolonged exposure to hostile environmental conditions that include but not limited to suboptimal temperature, UV irradiation, nutrient deprivation and biological interactions[5,139]. The VBNC state was first invoked by Xu *et al*.[140] who conducted survival studies of *Vibrio cholerae* and *E. coli* in fresh and marine water microcosms. The VBNC state was reported in *Campylobacter* spp. and many other enteric bacterial species such as *Salmonella*, *Shigella*, *Legionella* and *E. coli*[117,141].

Non-culturability in *Campylobacter* spp. has been attributed, in part, to exposure to elevated temperature[117,120], also nutrient depletion, salinity, and aeration among other factors that may induce campylobacters to undergo the VBNC state[142,143]. *Campylobacter* spp. in VBNC state may undergo morphological and physiological changes in response to environmental stressful conditions. Morphological changes may include the formation of elongated spirals; rods and coccoid cells, whereas reduction in ATP concentration and loss of cell membrane fatty acids among the physiological changes that exhibited by campylobacters in VBNC state[117,120]. Hudock *et al.*[144] suggested *C. jejuni* in VBNC usually form the spiral-shaped forms, the formation of coccoid cells, however was associated with degradation of DNA and of the cells. *Campylobacter* spp. in VBNC state may be able to be resuscitated; this was shown by various studies that reported the colonization of suckling mice, the gut of rats, 1-week-old chicks and fertilized chicken eggs by *Campylobacter* spp. in VBNC state[145-148]. Indeed, Baffone *et al*.[149] were able to resuscitate *C. jejuni*, after up to 142 days as VBNC cells in artificial sea water, by passage through mouse intestine. The ability of *C. jejuni* in VBNC state to be resuscitated in their host may potentially suggest its ability to retain their virulence and thus their capability of causing infections. Although VBNC forms of *Campylobacter* spp. can be resuscitated that could lead to infection, it is not clear if these cells are able to cause infection without prior resuscitation[5].

The VBNC phenomenon may still remain unclear, nonetheless, from a public health stand point its relevance falls into two areas: *i.e.* the ability of VBNC cells to cause infection and the monitoring of VBNC enteric pathogens in environmental samples by conventional culture methods[5].

6. Concluding remarks

Campylobacter species remain the leading cause of gastroenteritis worldwide. The consumption of untreated water, unpasteurized milk and undercooked meat are the main sources of infection. Handling domestic and wild fauna may also pose health risks of acquiring campylobacteriosis. *Campylobacter* spp. are common inhabitants of the intestinal tract of warm-blooded animals, particularly free-living birds and food animals. *Campylobacter* spp. are ubiquitous in the environment, with wild birds, sewage effluents and rain-related runoff being the major sources of the pathogen in the environment. Wild birds were implicated in the contamination of drinking water supplies, milk bottles, and recreational water with campylobacters which resulted in outbreaks of campylobacteriosis. Untreated sewage effluents were also an important source of campylobacters in surface waters, particularly fresh and marine bathing sites. Treatment of sewage effluents and sludge was found to reduce the numbers of campylobacters, and primary treatment can reduce the numbers of campylobacters less than tertiary treatments than completely eliminate campylobacters in sewage effluents and sludge. *Campylobacter* spp. are able to survive in aquatic environments and food, low temperatures and the formation of biofilm favors the survival of the pathogen in diverse environments, therefore control strategies of the organisms within the food chain need to continuously developed. In order for campylobacters to survive outside the host, it might enter the viable but non culturable state, which may have major relevance to public health, as they may be able to cause infection, yet may not be detectable during the monitoring of samples by conventional culturing methods.

Conflict of interest statement

We declare that we have no conflict of interest.

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