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MINIREVIEW

Does *Campylobacter jejuni* Form Biofilms in Food-Related Environments?

Amy Huei Teen Teh, Sui Mae Lee, Gary A. Dykes*

School of Science, Monash University, Bandar Sunway, Selangor, Malaysia

***Campylobacter jejuni* is one of the most frequent causes of bacterial gastrointestinal food-borne infection worldwide. This species is part of the normal flora of the gastrointestinal tracts of animals used for food production, including poultry, which is regarded as the primary source of human *Campylobacter* infections. The survival and persistence of *C. jejuni* in food processing environments, especially in poultry processing plants, represent significant risk factors that contribute to the spread of this pathogen through the food chain. Compared to other food-borne pathogens, *C. jejuni* is more fastidious in its growth requirements and is very susceptible to various environmental stressors. Biofilm formation is suggested to play a significant role in the survival of *C. jejuni* in the food production and processing environment. The aims of this minireview were (i) to examine the evidence that *C. jejuni* forms biofilms and (ii) to establish the extent to which reported and largely laboratory-based studies of *C. jejuni* biofilms provide evidence for biofilm formation by this pathogen in food processing environments. Overall existing studies do not provide strong evidence for biofilm formation (as usually defined) by most *C. jejuni* strains in food-related environments under the combined conditions of atmosphere, temperature, and shear that they are likely to encounter. Simple attachment to and survival on surfaces and in existing biofilms of other species are far more likely to contribute to *C. jejuni* survival in food-related environments based on our current understanding of this species.**

Thermophilic *Campylobacter* species are one of the most frequent causes of bacterial food-borne gastrointestinal infection worldwide. Of the 18 species of *Campylobacter* described, 85% of human campylobacteriosis cases are caused by *Campylobacter jejuni* (1, 2). Campylobacters are Gram-negative, non-spore-forming, curved-to-spiral rod-shaped bacteria which belong to the family *Campylobacteraceae* (3). The symptoms of campylobacteriosis generally entail diarrhea, fever, and abdominal pain but may also include neuropathies such as Guillain-Barre and Miller-Fisher syndromes (4, 5).

C. jejuni is part of the normal flora of the gastrointestinal tracts of a number of domestic and wild mammal and avian species (6, 7). Of these animals, poultry is generally regarded as the primary source of human *C. jejuni* infection (8). Contamination of retail poultry with *C. jejuni* occurs from the gastrointestinal tract of the animal during processing (9). Consumption of undercooked poultry and raw milk (10, 11), as well as consumption of other foods that have been cross contaminated by animal products, is strongly associated with *C. jejuni* infections (12).

Compared to many other food-borne pathogens, such as Shiga-toxigenic *Escherichia coli* and *Salmonella enterica*, *C. jejuni* is more fastidious in its growth requirements. Specifically, it requires a reduced oxygen atmosphere (5% oxygen, 10% carbon dioxide, and 85% nitrogen) to grow (13). *C. jejuni* is also unable to grow at temperatures below 30°C and is susceptible to various environmental and food processing-induced stressors, such as osmotic stress, elevated temperature, and pH (3, 14, 15). These properties theoretically make *C. jejuni* unsuitable for survival outside the host in natural aerobic environments or in the food chain (1, 16). In reality, however, *C. jejuni* is widely spread in the environment and can be readily isolated from food, water, and other sources (14, 17). It is not clear as to how *C. jejuni* overcomes these apparent disadvantages to survive in the environment and the food chain and then goes on to cause disease. Reports on research undertaken on this apparent paradox have suggested that biofilm formation may play a significant role in survival of *C. jejuni* in the environment (18, 19).

The formation of biofilms by *C. jejuni* has been studied for a number of years under laboratory conditions, but these studies are disparate, and many studies use only one strain or very specific experimental conditions. Unlike biofilms formed by many other species of bacteria, which are often architecturally complex structures, there is little information available suggesting the formation of a specific and consistent biofilm morphology by *C. jejuni* as a species. This raises the question as to whether *C. jejuni* (as a species) forms biofilms as a survival mechanism in the environment or if it just attaches to surfaces (or other biofilms) in a far more passive way. This minireview addresses this issue in general and more specifically examines the extent to which studies of *C. jejuni* biofilm formation under laboratory conditions contribute to our understanding of their survival in the environment.

DEFINITION OF BIOFILMS

Biofilms are usually defined as monospecies or multispecies structured communities of microbial cells enclosed in a self-produced polymeric matrix and adherent on inert or living surfaces or interfaces (solid-liquid, liquid-liquid, or liquid-gas) (20). This definition includes microbial aggregates and floccules as well as adherent populations within the pore spaces of porous media (21). Biofilms protect the bacteria from various environmental stres-

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Address correspondence to Gary A. Dykes, gary.dykes@monash.edu.

* Present address: Gary A. Dykes, School of Chemistry, Monash University, Clayton, Victoria, Australia.

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sors, increase their resistance toward many antimicrobial agents (such as antibiotics), and provide protection against host defense mechanisms (20, 22). In the food industry, biofilms formed on food processing surfaces protect the bacteria from cleaning and sanitation procedures, which may in turn lead to the contamination of food products and increase the potential of the bacteria to cause diseases if they are pathogenic (23, 24). This definition does not include single adherent, nongrowing, or metabolically damaged cells that may attach to surfaces by purely physiochemical processes, although such cells may subsequently go on to form biofilms if environmental conditions are suitable.

C. JEJUNI BIOFILMS ON ABIOTIC SURFACES

As is the case for other bacterial species, biofilm formation by *C. jejuni* on food contact surfaces, such as stainless steel and plastics, typically found in food processing environments or in poultry house water systems, may contribute to the persistence and survival of *C. jejuni* outside the host under environments that are detrimental to them. These biofilms may act as a source of contamination and contribute to the high number of human *C. jejuni* infections (18, 19, 23). A clear understanding of how, and to what extent, *C. jejuni* forms biofilms on abiotic surfaces is important in order to develop strategies to prevent the contamination of food products by these bacteria on food processing surfaces. Investigations into the ability of *C. jejuni* to form biofilms on abiotic surfaces in the laboratory have been conducted (4, 19, 25–30), but compared to most other bacterial food-borne pathogens, these studies are relatively few and still in their infancy. Even fewer studies that support the premise that *C. jejuni* can form biofilms on abiotic surfaces under typical environment conditions have been conducted (31).

C. jejuni can form monospecies biofilms when grown *in vitro* in culture media on a variety of surfaces, including stainless steel (19, 25, 26, 32), glass (4, 19, 30), nitrocellulose membranes (19), and various plastic surfaces (25, 27, 28, 31, 33, 34). Not all studies confirm this pathogen's ability to form biofilms on all surfaces, and findings to the contrary are not uncommon. For example, while a study carried out by Reeser et al. (31) showed that *C. jejuni* attached well to, and was a good biofilm former on, hydrophobic surfaces such as plastics found in watering systems, other studies have reported that *C. jejuni* was unable to attach to polystyrene (4, 26). These differences might be due to strain variations or merely differences in the experimental setups used in the different studies.

Most studies investigating *C. jejuni* biofilms have used the crystal violet staining method to detect and quantify biofilms formed (26, 31, 34–36). This method is one of the most commonly used methods to study biofilms and is based on the principle that crystal violet binds to negatively charged surface molecules that are found on both the bacteria and the extracellular matrix of the biofilm (37, 38). Other techniques, such as cell enumeration, have also been used to study *C. jejuni* biofilms (25, 31). Both the crystal violet staining and cell enumeration methods quantify the biofilms based on the attachment of the cells to the surfaces but do not demonstrate the presence or nature of specific biofilm structures.

To date, a very limited number of studies have shown microscopic evidence that *C. jejuni* is able to form architecturally complex biofilms as reported for many other bacterial species. Direct imaging of *C. jejuni* biofilms using confocal laser scanning microscopy (CLSM) or scanning electron microscopy (SEM) have been used in some studies (4, 19, 26, 32, 39). These studies have demonstrated that *C. jejuni* biofilms may occur in three different forms in liquid culture: (i) cells attached to inert surfaces; (ii) flocs (aggregates of cells floating in the liquid); and (iii) pellicles (aggregates of cells formed at the air-liquid interface) (4, 19, 40). Joshua et al. (4) established that *Campylobacter* bacteria in the three forms mentioned above were connected via extracellular matrix using SEM. They proposed that these three forms represent true biofilms, since they all have similar architecture to one or other of the stages of biofilm formation. Specifically, attached cells represent the initial step in biofilm formation, flocs resemble biofilms in structure, and pellicles share a similar architecture to attached biofilms with the bacterial cells connected via an extracellular polymetric matrix (EPM) (4, 41). Studies carried out by Kalmokoff et al. (19) also demonstrated that repeated transfer of surfaces inoculated with *C. jejuni* into fresh medium lead to the development of more-complex adherent cell layers along with the presence of an EPM. They suggested that this adherent cell layer represents a mature biofilm (19). It should be noted, however, that only one strain of *C. jejuni* (*C. jejuni* 11168) was used in the latter study, and this limits our ability to draw general conclusions about the species from the data. This is because other studies have established that not all the *C. jejuni* strains investigated were able to form biofilms under the same conditions (4, 26, 31, 36). It is clear that more *C. jejuni* strains need to be examined to determine the nature of biofilm formation in this species as a whole.

While *C. jejuni* has been shown to form biofilms, the extent of biofilm formation is much lower than those formed by other microorganisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus simulans*, and *Salmonella enterica* serovar Agona (36). Most studies investigating biofilm formation by *C. jejuni* on abiotic surfaces have been carried out under microaerophilic conditions *in vitro* at temperatures above 30°C, which do not represent the conditions these bacteria are likely to face in the environment. The ability of *C. jejuni* to form biofilms in the food processing environment has not been established by these studies.

C. JEJUNI BIOFILMS UNDER AEROBIC CONDITIONS

While most studies of biofilm formation by *C. jejuni* have been carried out under microaerobic conditions, some studies have been undertaken to examine biofilm formation by *C. jejuni* under aerobic conditions. Studies carried out by Asakura et al. (34) and Reuter et al. (35) showed that biofilm formation by *C. jejuni* was increased under aerobic conditions. They suggested that *C. jejuni* forms biofilms in response to oxidative stress. This may protect them from lethal conditions by providing a microaerobic environment that supports their survival and growth. Both these studies, however, were carried out using the same *C. jejuni* strain (*C. jejuni* NCTC11168), and the ability of other strains to do the same has not been established. In addition, another study (31) found that biofilm formation by *C. jejuni* was enhanced under lower oxygen tension conditions, which favored the growth of *C. jejuni*, and was inhibited under aerobic conditions, which reduced or eliminated growth. Further studies on this aspect of biofilm formation are required, since many questions remain about the ability of *C. jejuni* to form biofilms under environmental oxidative stress.

C. JEJUNI BIOFILMS AT DIFFERENT TEMPERATURES

The survival of *C. jejuni* outside its host is affected by temperature (25, 28, 30, 42). Chan et al. (42) found that survival of planktonic *C. jejuni* is enhanced at low temperatures. Dykes et al. (30) found that *C. jejuni* grown as planktonic cells and as biofilm cells survived longer at lower temperatures (4°C and 10°C) than at higher temperatures (25°C and 37°C) under stress conditions. The study of Hanning et al. (25) showed that survival of culturable *C. jejuni* cells in biofilms kept at 32°C was longer than survival of culturable planktonic cells. Survival of culturable *C. jejuni* cells in biofilms kept at 10°C, on the other hand, was either not different or was lower than the survival of culturable planktonic cells. The ability of *C. jejuni* to form biofilms may be different at different temperatures. For example, Reeser et al. (31) showed that biofilm formation by *C. jejuni* was higher at 37°C than at 25°C. Studies on the effect of temperature on biofilm formation by *C. jejuni* are, however, limited. Most studies on *C. jejuni* biofilms thus far have been carried out at 37°C (4, 32, 34–36). This temperature is not suitable to evaluate biofilm formation by *C. jejuni* of poultry origin or under food processing conditions, as this temperature is relevant only for mammals but is too low for poultry (42°C) and too high for food processing (generally less than 25°C).

C. JEJUNI BIOFILMS UNDER FLOW CONDITIONS

In most studies described above, biofilms of *C. jejuni* were grown under static conditions with little or no shear force applied to them. These growth conditions are different from those found in the water supplies and plumbing systems of animal husbandry facilities and processing plants where *C. jejuni* biofilms have been suggested to form (11, 18, 31). It is therefore important to investigate *C. jejuni* biofilm formation under flow conditions in order to understand the mechanisms that allow biofilm formation under dynamic conditions in the environment.

Studies carried out by Joshua et al. (4) reported that *C. jejuni* strains which strongly attached to glass in standing cultures did not attach to glass or any other surfaces (cellulose acetate filters, tissue culture flasks, or 24-well tissue culture plates) when they were grown under moderate shaking (80 to 100 rpm). These authors noted that *C. jejuni* developed into a biofilm by forming aggregates in the culture when they were grown at lower shaking rates (50 rpm). This may be due to higher oxygen levels in the culture medium under more vigorous shaking conditions that are unfavorable for the growth and survival of *C. jejuni* cells, resulting in them being unable to attach and form biofilms under shaking conditions. In addition, these same authors (4) also reported that *C. jejuni* failed to form biofilms in a modified Robbins device, a system which has been widely used in other studies (43), with a typical flow rate of 300 ml h^{-1} or even substantially lower at 10 ml h^{-1}. Similar observations were made by Ica et al. (39) who reported that monoculture *C. jejuni* biofilms were unable to persist at higher flow rates (60 to 150 ml h^{-1}). These authors did report that *C. jejuni* biofilms can persist at lower flow rates (45 ml h^{-1}) (39). The difference in the ability of *C. jejuni* to form biofilms at low flow rates in these two studies may be due to differences in methodology. The first study investigated the ability of *C. jejuni* to establish biofilms under flow conditions, while the latter allowed *C. jejuni* to attach and form biofilms prior to subjecting them to flow conditions in order to investigate the effect of flow rate on *C. jejuni* biofilm structure. Importantly, both of these studies indicated that *C. jejuni* was unable to form biofilm under flow conditions and the preformed biofilm was unable to persist at higher flow rates. This indicated that the structure of biofilms formed by *C. jejuni* in monoculture was fragile. This is contrary to previous findings which observed the presence of *C. jejuni* in biofilms in the water supply and plumbing systems of animal husbandry facilities and animal processing plants where constant shear forces were present (18, 31). One possible explanation for these observations might be the ability of *C. jejuni* to form mixed biofilms with other bacterial species in the environment.

C. JEJUNI IN MIXED-SPECIES BIOFILMS

Biofilms can consist of single or multiple microbial species, but in nature mixed-species biofilms predominate in most environments (44). Previous studies have shown that *C. jejuni* is a poor biofilm initiator and monospecies *C. jejuni* biofilms form only under specific growth conditions that support the growth of *C. jejuni* (4, 32). Since *C. jejuni* is susceptible to the conditions prevalent outside its hosts, the possibility that *C. jejuni* acts as a primary colonizer for biofilm formation in the environment, such as poultry processing surfaces, is low. Hanning et al. (25) suggested that *C. jejuni* might act as a secondary colonizer and be incorporated into preestablished biofilms and persist in multispecies biofilms (25). Other studies have proposed a similar concept of one bacterial species acting as a primary colonizer and promoting biofilm formation by another species. Several other studies have also proposed that under conditions that inhibit its growth, *C. jejuni* is able to survive by forming mixed biofilms with other bacterial species (10, 18, 25, 28, 29, 32).

Teh et al. (36) investigated the ability of *C. jejuni* to form biofilms in mixed microbial populations consisting of five different bacteria, *P. aeruginosa*, *E. coli*, *E. faecalis*, *S. simulans*, and *S.* serovar Agona. The authors were able to recover *C. jejuni* cells from most of the mixed-species biofilms in their study, indicating that *C. jejuni* was able to attach and survive in the biofilms. The number of cells recovered varied between the different mixed microbial populations with the highest recovery from biofilms that included either *E. faecalis* and/or *S. simulans*. Other studies have shown similar results for the attachment of *C. jejuni* to *E. faecalis* and/or *S. simulans* biofilms (32, 45, 46). It has been suggested that these microorganisms that originate from poultry sources may provide a suitable environment for the survival and growth of *C. jejuni* in poultry processing plant environments (47, 48). Although *C. jejuni* cells were recovered from most of the mixed-species biofilms in their study, Teh et al. (36) found that most of the mixed-species populations that contained *P. aeruginosa* did not harbor *C. jejuni* cells. This is in contrast to the results obtained from other studies which showed that *C. jejuni* was able to coexist and form a mixed-species biofilm with *P. aeruginosa* (39, 49). The difference in the results observed might be due to strain variation or different experimental conditions used in the studies.

In a biofilm, bacterial cells are embedded in an extracellular matrix in close proximity to each other, which facilitates genetic exchange and sharing of nutrients, enzymes, and secondary metabolites within these communities (50). These characteristics of mixed-species biofilms may be advantageous to *C. jejuni* which has a limited genetic complement for biosynthesis of essential metabolites due to its relatively small genome (25, 51). Parkhill et al. (51) found that the genome of *C. jejuni* has very few genes for degradation of carbohydrates or amino acids but appears to have a large number of genes for transport systems. Since *C. jejuni* is

unable to utilize many carbohydrates as carbon or energy sources, it may be dependent on secondary metabolites produced by other bacteria in the mixed-species biofilm. Furthermore, the presence of genes for transport systems is suggested to play an essential role in the uptake of amino acids not synthesized by *C. jejuni* but produced by other bacteria or found in the environment of the mixed-species biofilms (25).

Several studies have shown that *C. jejuni* survived better in mixed-culture biofilms than in monospecies biofilms, especially under environmental conditions that did not favor its growth. Ica et al. (39) established that *C. jejuni* was unable to be cultured from a monospecies biofilm grown under aerobic conditions but were alive (according to live/dead staining) indicating that the cells had entered a "viable but not culturable" (VBNC) state. On the other hand, *C. jejuni* cells were able to be cultured from a mixed-species biofilm grown under the same conditions, suggesting that the *C. jejuni* cells are more protected from environmental stress, in particular oxidative stress, in a mixed-species biofilms compared to monospecies biofilms. In addition, Hilbert et al. (49) reported that *C. jejuni* coexisting with *Pseudomonas* spp. showed prolonged survival under aerobic conditions. The authors suggested that this might be due to metabolic commensalism in which *Pseudomonas* spp. consumed the oxygen and protected *C. jejuni* from atmospheric oxygen tension (49). This is supported by a study carried out by Ica et al. (39) which showed that the dissolved oxygen concentration in a mixed-species biofilms formed by *C. jejuni* and *P. aeruginosa* concomitantly was approximately zero at the end of the fifth day. This suggested that oxygen was consumed by *P. aeruginosa*, creating a favorable environment for the survival and growth of *C. jejuni*.

Ica et al. (39) also reported that the biofilm structures of the monoculture and mixed-culture biofilms of *C. jejuni* are significantly different, because while mixed-culture biofilms increase in size over time, the monoculture biofilms detach from the substrate until the biofilm structure reaches a pseudo-steady state. Furthermore, these authors also compared the effect of flow rate on *C. jejuni* monoculture and mixed-culture biofilm structures. As mentioned previously, monoculture *C. jejuni* biofilms could persist under a lower flow rate (0.75 ml/min) but not under higher flow rates (1 to 2.5 ml/min). In contrast, it was reported that mixed-culture *C. jejuni* biofilms could persist at higher flow rates (2.5 ml/min), indicating that mixed-culture biofilms are more stable and robust than monoculture biofilms are (39). These findings suggested that mixed-culture biofilms might be a possible explanation for the survival and persistence of *C. jejuni* in poultry processing plants where constant shear forces were present.

In addition to the factors discussed above, other factors such as nutrient availability and salinity may also affect biofilm formation by *C. jejuni*. For example, a study carried out by Reeser et al. (31) found that *C. jejuni* formed more biofilms in Mueller-Hinton broth than in other nutrient-rich media such as brucella and Bolton broths. Reeser et al. (31) also found that an increase in the concentration of NaCl and other osmolytes, such as glucose and sucrose, resulted in a significant decrease in biofilm formation by *C. jejuni*. Genetic background has been shown to affect biofilm formation by *C. jejuni*. Studies have shown that biofilm formation by *C. jejuni* was lower in strains defective in, for example, a putative flagellar protein (FliS) and in a phosphate acetyltransferase (Cj0688) (4). Strains with a mutated CsrA (carbon starvation regulator) gene (33) or deficient in flagella (*flaAB* mutants) (31) also produce less biofilm. Few studies have been carried out to investigate the effects of these factors on biofilm formation by *C. jejuni* under the different conditions of concern in this study and were therefore not discussed in detail.

CONCLUDING REMARKS

This minireview of the available literature on biofilm formation by *C. jejuni* exposes a number of issues which the research community needs to address to increase the relevance of studies on the survival of this pathogen in food-related environments. These issues are highlighted in Table 1, which compares the main methodological characteristics of the key studies in this area. As discussed above, the lack of evidence is based on experimental issues related to the following: (i) atmosphere; (ii) biofilm quantification; (iii) monospecies versus mixed-species biofilms; (iv) number of strains; (v) temperature; and (vi) shear. Overall, it is clear that some strains of *C. jejuni* form biofilms on some surfaces/interfaces under some growth conditions. It is equally clear that none of the studies, singly or together, provide evidence for the formation of biofilms (as usually defined) in the environment despite most suggesting the relevance of their studies to food (and usually poultry) processing.

Experimental growth temperature can be used as an illustrative example of how studies might improve their relevance in two ways. First, the presence of *C. jejuni* in food-related environments is due to their growth in the host and contamination of food and processing surfaces through animal feces either directly or during the slaughter process. Their growth temperature in experiments (to prepare inocula for biofilm formation work) should be relevant to the host of interest (37°C for most mammals and 42°C for most birds), as these growth conditions are strongly relevant to their subsequent behavior and survival (52). Many existing studies use one temperature but suggest its relevance to an inappropriate host. Second, after leaving the host, except in unusual circumstances, *C. jejuni* should not be able to grow in the environment, not necessarily primarily because of the atmosphere (in which some growth may occur), but because temperatures are unlikely to be above 30°C (below which the bacteria will not grow). Studies should therefore examine biofilm formation (using inocula grown at the appropriate temperature) under conditions which combine temperatures (below 30°C) and all other conditions relevant to the food processing environment of interest if they wish to make claims about the value of the study to these environments. It can be asserted that under these conditions biofilm formation *per se* is very unlikely to occur. Instead *C. jejuni* cells are likely to simply attach to food, abiotic surfaces, and biofilms of other species in a relatively passive way. This attachment should not be regarded as an initial step in biofilm formation, since subsequent growth cannot occur, but it is still likely to enhance their survival, particularly in the case of biofilms of other species which may reduce their exposure to stressors. Cells of *C. jejuni* in the viable but nonculturable (VBNC) state have been demonstrated to attach to stainless steel as effectively as viable cells do (53). Biofilms of other bacteria with *C. jejuni* attached to them in the environment may simply be delaying entry of the *C. jejuni* into the VBNC state by protecting them from stressors, rather than by forming biofilms with them. It is suggested, based on the current state of knowledge, that extrapolation from *C. jejuni* biofilms in the laboratory to those in food processing environments should not be made. Instead *C. jejuni* on surfaces (and in other biofilms) in these envi-

TABLE 1 Summary of key studies investigating biofilm formation by *C. jejuni* under different conditions

Study	Analysis of biofilm	Aerobic or microaerobic	Incubation temp (°C)	Mono-/multispecies	Static or flow conditions	No. of strains used	Main result(s)
Joshua et al. (4)	Observation of aggregates, pellicles, or attached biofilms on abiotic surfaces SEM-CLSM analysis of biofilms formed on glass	Microaerobic	37	Monospecies	Static, shaking, and flow conditions	17	Three distinct biofilms formed by *C. jejuni* (attached, flocs, pellicles) *C. jejuni* unable to form biofilms under shaking and flow conditions
Kalmokoff et al. (19)	SEM analysis of biofilm structures on nitrocellulose membranes, stainless steel, and glass fiber filters	Microaerobic	37	Monospecies	Static	1 (*C. jejuni* NCTC11168)	Multicellular layers consisting of EPM formed by *C. jejuni*, representing a mature biofilm
Asakura et al. (34)	Attachment on 24-well polystyrene plates and quantified by crystal violet staining	Aerobic and microaerobic	37	Monospecies	Static	1 (*C. jejuni* NCTC11168)	*C. jejuni* biofilm formation higher under aerobic than microaerobic conditions
Reeser et al. (31)	Attachment on 24-well polystyrene plates, quantified by crystal violet staining	Aerobic and microaerobic	37/25	Monospecies	Static	1 (*C. jejuni* M129)	Biofilm formation enhanced under conditions favoring growth (microaerophilic, 37°C) Biofilm formation inhibited by nutrient-rich media/high osmolarity
	Attachment on abiotic surfaces, quantified by cell enumeration	Microaerobic	37	Monospecies	Static	1 (*C. jejuni* M129)	*C. jejuni* can attach to hydrophobic and hydrophilic surfaces, with more biofilm formed on latter
Sanders et al. (32)	CLSM analysis of biofilms on stainless steel	Aerobic	37	Multispecies	Static	1 (*C. jejuni* RM1221)	*C. jejuni* formed biofilms on stainless steel when incubated with mixed bacterial populations
Hanning et al. (25)	Attachment to mixed-species preestablished biofilms on stainless steel chips, quantified by cell enumeration	Aerobic	10/23/32	Multispecies	Static	1 (*C. jejuni* 43431)	Preestablished biofilms facilitate *C. jejuni* attachment to surfaces
Gunther and Chen (26)	Attachment on abiotic surfaces, quantified by crystal violet staining SEM analysis of biofilms formed on glass	Microaerobic	42	Monospecies	Static	2 strains (*C. jejuni* 81-176 and RM1221)	Biofilm formation different for different strains
Reuter et al. (35)	Attachment on glass test tubes, quantified by crystal violet and Congo red staining Light microscopy analysis of biofilms formed on glass	Aerobic and microaerobic	37	Monospecies	Static	1 (*C. jejuni* NCTC11168)	*C. jejuni* biofilm formation higher under aerobic than microaerobic conditions
Teh et al. (36)	Attachment on 24-well polystyrene plates, quantified by crystal violet staining	Microaerobic	37	Mono- and multispecies	Static (gentle swirling)	20	*C. jejuni* can form mixed-species biofilms
Ica et al. (39)	Biofilm structures quantified by microscopy	Aerobic	25/37	Mono- and multispecies	Flow	1 (*C. jejuni* NCTC11168)	Monoculture *C. jejuni* biofilms unable to persist at higher flow rates Mixed-culture biofilms more stable and robust than monoculture biofilms

ronments should be referred to as attached or adherent until credible information to the contrary is available.

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