Journal of Microbiological Methods 95 (2013) 68–75

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Use of phages to control *Campylobacter* spp.

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ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 22 June 2013
Accepted 25 June 2013
Available online 3 July 2013

Keywords:
Phage therapy
Campylobacter
Biocontrol
Bacteriophages
Phage applications

ABSTRACT

The use of phages to control pathogenic bacteria has been investigated since they were first discovered in the beginning of the 1900s. Over the last century we have slowly gained an in-depth understanding of phage biology including which phage properties are desirable when considering phage as biocontrol agents and which phage characteristics to potentially avoid. *Campylobacter* infections are amongst the most frequently encountered foodborne bacterial infections around the world. Handling and consumption of raw or undercooked poultry products have been determined to be the main route of transmission. The ability to use phages to target these bacteria has been studied for more than a decade and although we have made progress towards deciphering how best to use phages to control *Campylobacter* associated with poultry production, there is still much work to be done. This review outlines methods to improve the isolation of these elusive phages, as well as methods to identify desirable characteristics needed for a successful outcome. It also highlights the body of research undertaken so far and what criteria to consider when doing *in-vivo* studies, especially because some *in-vitro* studies have not been found to translate into to phage efficacy *in-vivo*.

1. Introduction

Sebald and Véron first proposed the *Campylobacter* genus in 1963 and the number of members in the genus is constantly increasing due to improved isolation and detection methodologies (Butzler, 2004). There are currently 25 species in the *Campylobacter* genus (Man, 2011). They are typically microaerobic spiral-shaped Gram-negative cells, but have been found to also grow aerobically and anaerobically. They are thermophilic and have an optimum growth temperature of 42 °C but can grow at 30 °C. Their reservoirs are warm-blooded animals such as cattle, sheep, pigs, poultry, wildlife and domestic pets. Animal carriers are generally asymptomatic, but the species *C. fetus* can cause diarrhea and aseptic abortions in large animals (Moore et al., 2005). Most human campylobacteriosis are due to *C. jejuni* (90%) with a few due to *C. coli* (10%) (Wilson et al., 2008). Other species such as *C. upsaliensis*, *C. lari*, and *C. fetus*, have also been associated with human diarrhea, particularly in the developing world (Coker et al., 2002; Man, 2011).

In the United States, there are 9.4 million reported cases of foodborne illnesses each year. The third most prevalent bacterial pathogen causing 9% of those cases are attributed to *Campylobacter* species (Scallan et al., 2011) with an estimated annual cost of illness to be $1.7 billion (Hoffmann et al., 2012). However, in Europe campylobacteriosis is the most common foodborne bacterial illness and estimates of the true incidence rate for *Campylobacter*-associated infections is 9.2 million, with a total annual cost of €2.4 billion (EFSA, 2011). Underreporting from such infections could be due to its self-limiting nature, which presents as an acute gastrointestinal illness resulting in diarrhea, fever and abdominal cramps with a mean duration of six days (Butzler, 2004; Man, 2011). Campylobacteriosis is the most frequently reported zoonotic disease in the EU, with poultry meat being the major source for human cases (EFSA, 2011). In the U.S. *Campylobacter* species are found to cause the most illnesses attributed to animal contact, although they are second to nontyphoidal *Salmonella* as pathogens causing hospitalization or death (Hale et al., 2012).

Up to 30% of human cases are due to handling, preparation and consumption of raw or undercooked poultry, although the routes of transmission from chickens to humans are not well understood (EFSA, 2011). A linear relationship has been found between the prevalence of *Campylobacter* in broiler flocks and the risk to public health through quantitative risk assessment studies of interventions in the primary production in four European countries (Nauta et al., 2009). A 90% reduction in the risk to public health could be obtained by reducing the number of *Campylobacter* in broiler intestines by 3 Logs, and even a 1 Log reduction on the carcasses would reduce the risk to between 50 and 90% (EFSA, 2011).

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0167-7012
http://dx.doi.org/10.1016/j.mimet.2013.06.024

2. Approaches to reducing *Campylobacter* in poultry

Preventative measures during primary production to prevent *Campylobacter* from being transmitted to a flock requires adequate biosecurity implementations such as use of modern housing that is well maintained and with limited access. Boot dips, and fly screens have both been found to reduce the risk of flock infection. However, in order to maximize the reduction of *Campylobacter*-positive flocks at the farm level, complimentary non-biosecurity measures, such as thoroughness of cleaning and disinfecting the poultry house between flocks, are also necessary (Newell et al., 2011). Reducing flock susceptibility to infection by using appropriately treated water for drinking, reducing slaughter age and discontinuing thinning are important strategies at preventing flock colonization by *Campylobacter*, or at least delaying its onset until close to slaughter (Newell et al., 2011). At the processing plant, the various stages of processing chicken carcasses (i.e. chilling, washing, defeathering, scalding and evisceration) have shown a limited effect at reducing the presence of *Campylobacter* (Guerin et al., 2010). Post-slaughter — only irradiation or cooking on an industrial scale can reduce the risk by 100%. Freezing the carcasses for 2–3 weeks can help reduce the risk by >90%, whereas freezing for 2–3 days will reduce the risk between 50 and 90%, similar to carcass decontamination using hot water, lactic acid, acidified sodium chlorite or trisodium phosphate (Whyte et al., 2003; Riedel et al., 2009; Sampers et al., 2010; EFSA, 2011; Meredith et al., 2013).

One particular strategy that has been investigated for its potential application to reduce the load of *Campylobacter* in chickens, from the primary intervention stage through to the packaging stages of processed carcasses, is the use of bacteriophages (Atterbury et al., 2003b; Goode et al., 2003; Loc Carrillo et al., 2005; Wagenaar et al., 2005). Bacteriophages (also known as phages) are bacterial viruses that can only lyse targeted bacterial cells.

The specificity of phages to target bacteria is dependent on cell surface receptors such as outer membrane and lipopolysaccharide proteins as well as flagella components (Lindberg, 1973; Scott et al., 2007a; Holst Sorensen et al., 2011). Once the phage is irreversibly attached, its DNA is ejected into the cell and takes over the host's DNA replication, transcription and translation processes. Phage components are manufactured by the bacterium and then assembled to make progeny phage, which exit the cell with the aid of lysins. These 'new' phages are then capable of infecting other target cells nearby.

3. Isolation and characterization of *Campylobacter* phages

The ideal phages for use as therapeutic or biocontrol agents on pathogenic bacteria such as *Campylobacter* must meet a few criteria before being considered as suitable candidates. They must be obligately lytic and therefore only capable of infecting bacterial cells and producing progeny phage without the capacity to integrate into the bacterial genome or transduce bacterial genes from one cell to another (Canchaya et al., 2003). These therapeutic phages should also possess physical characteristics that will facilitate their survival in the environments they will be applied to, i.e. stable at high temperature (such as the body temperature of live chickens, 42 °C), stable at low pH (such as that found in the chicken gut, pH 2–4) and have well-characterized lytic activity against target bacteria but not commensals (Loc Carrillo, 2005; Hansen et al., 2007).

3.1. Isolation

The best sources to isolate phage are from environments where the host is highly prevalent. *Campylobacter* is ubiquitous in temperate environments but favors the intestine of all avians, where they colonize the gut as a commensal organism (Newell and Fearnley, 2003). By isolating the *Campylobacter* strains from the same location as their phage, there is a higher probability of having a host susceptible to the phages sought. Failing the possibility of isolating 'native' hosts, possessing a highly susceptible strain such as *C. jejuni* NCTC 12662, which is known to be sensitive to most *C. jejuni* and *C. coli* phage isolates, is an alternative solution (Connerton et al., 2004; Loc Carrillo, 2005; Owens et al., 2012).

Phage isolation begins with the collection and preparation of the environmental sample. *Campylobacter* phages have been isolated from retail poultry, feces and intestines of chickens and ducks, abattoir effluents, human feces, pig and poultry manure, as well as sewage (Grajewski et al., 1985; Salama et al., 1989; Atterbury et al., 2003a; Connerton et al., 2004; El-Shibiny et al., 2005; Hansen et al., 2007; Loc Carrillo et al., 2007; Owens et al., 2012). However, to date no *Campylobacter* phages have been recovered from cattle, rabbit and sheep feces, farm pasture soil, or water samples (Grajewski et al., 1985; Bigwood and Hudson, 2009).

A modified procedure by Salama and colleagues is particularly useful at isolating *Campylobacter* phages. Phages are eluted from solid matrix samples (e.g., feces and intestinal content) by suspending the material (1:10) in a suitable buffered solution such as Salt-Magnesium (SM) buffer. The suspension is then centrifuged to remove debris and the supernatant transferred to a new tube and centrifuged at higher speeds to remove most bacteria. Finally, the resulting supernatant is then filtered through a 0.2 μm pore-size (low-protein binding) filter to remove any remaining bacteria. The use of chloroform is not recommended due to some phages being sensitive to the chemical. Vortexing of the samples should also be limited as phages with long tail fibers may be physically damaged and become uninfective. The filtrate is then ready to be applied as (10 μL) spots onto lawns, or (100 μL) mixed with the bacterial culture to make pour plates with the target *Campylobacter* strains (Salama et al., 1989). This method is simple and takes three days to complete.

Preparation of the target bacteria is also an important step towards isolating *Campylobacter* phage. *Campylobacter* cells should be harvested in brain heart infusion (BHI) broth, supplemented with 10 mM $MgSO_4$ and 1 mM $CaCl_2$ since the presence of these cations are necessary for a number of phage types to attach to their cell surface receptors. The use of selective media such as NZCYM broth with 1.5% select agar + 10 μg/mL vancomycin can be useful when trying to isolate phages from fecal or other heavily contaminated samples. Bacterial lawns using known highly susceptible *Campylobacter* strains can increase the likelihood of isolating phages, as is using strains isolated from the same environmental source. All lawn plates should be incubated for 24 h at 42 °C under microaerobic conditions. Any plaques seen are then cored-out with the aid of a sterile 1 mL pipette tip and suspended into SM buffer. Single plaques should be propagated three times to ensure that the phage isolates represent a single clone.

An enrichment method can be used to amplify very low numbers of phage present in a sample. For an outline of the method, see Carvalho et al., 2010a. In brief this method involves using a mixture of target/indicator *Campylobacter* strains instead of a single strain. Filtered samples can then be added to the exponentially growing *Campylobacter* mixture, allowing any phage present to propagate in any susceptible host, and making them easier to be detected when spotting onto susceptible bacterial lawns.

When searching for *Campylobacter* phage, it is important to consider the predator–prey relationship that ties phage to their host. Phage replication is dependent on a host density threshold (Payne and Jansen, 2003) and if the host population is low the probability of isolating phage is significantly reduced. This correlation was seen during a survey of broiler houses in the UK between January 2001 and June 2002. No phages were isolated when *Campylobacter* was not detected in the chicken excreta and cloacal swab samples collected (Loc Carrillo, 2005). Of the 798 samples from 12 different farms, collected between one to four times per farm over the 18-month period, 71% were positive for *Campylobacter* and only 45% contained detectable

phages. Another smaller survey by Atterbury et al. (2005) testing 205 broiler chickens from 90 flocks belonging to 22 U.K. farms between August and September 2002, managed to isolated *Campylobacter* from 63% of cecal samples (recovery was 10^2 to 10^7 colony forming units (CFU)/g of cecal content). However, only 20% of the chickens harbored *Campylobacter* phage present in a wide range of phage titers (recovery was 10^2 to 10^7 plaque forming units (PFU)/g of cecal content). Interestingly, El-Shibiny et al. (2005) found 51% of the 37 *Campylobacter*-positive organic birds tested harbored *Campylobacter* phage (recovery was 10^2 to 10^6 PFU/g of cecal content), indicating that birds with more access to the environment had a higher colonization rate.

3.2. Characterization

Once isolated and purified, phages must undergo a variety of genotypic and phenotypic tests necessary to identify them as suitable candidates for use as biocontrol agents.

3.2.1. Lytic spectra

Determining the lytic activity of a phage is the most common characterization, because it can help determine if the phage should be tested further. Phages possessing broad lytic spectra are often highly desirable, although phages capable of lysing bacterial strains that are less susceptible to a wide variety of phages are also deemed beneficial.

Lytic activity of a *Campylobacter* phage should be assessed with a panel of reference *Campylobacter* strains that include *C. jejuni* and *C. coli*, as well as wild-type isolates that reflect the environment under study (Hansen et al., 2007). Ideally these bacterial isolates will be well-characterized, either by phenotypic (e.g. serotyping, or phage typing) or genotypic methods (e.g. Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), or Restriction Fragment Length Polymorphism (flaA-RFLP)), prior to using in the panel. A high-throughput and convenient method to test host susceptibility against several phages simultaneously is the spot plate assay where a small volume (i.e. 10 μL) of phage suspension of known titer (i.e. *c.* 10^7 PFU/mL) is spotted onto the test bacterial lawn(s). Susceptible bacteria will be lysed around the spot and a visible clearing of the lawn will be seen, with sometimes only individual plaques seen. However, results should be interpreted carefully and if possible, a negative and positive control should be included in the assay. As a positive control, a well-characterized phage that produces a defined type of lysis on the host strain is recommended. This technique is similar to that used for phage typing of bacterial strains (Frost et al., 1999). For a more quantitative approach, the relative efficiency of plating (EOP) method could be undertaken (Kutter, 2009). This method could be used on the phage(s) that resulted in a clearing of the lawn using the spot plate assay. The phage suspension would be serially diluted and all its dilutions spotted onto a lawn of its propagation host as well as other susceptible bacterial strains. The resulting titers would be compared against each other to determine how virulent the phage is towards the different strains.

3.2.2. Morphological characteristics

A slightly more technical but relatively fast characterization method commonly used is determining the structure of phages. Phages can be examined using transmission electron microscopy (TEM) (Ackermann, 2007) and more recently using atomic force microscopy (Kuznetsov et al., 2011). It is advisable to have as high a titer of phage suspension as possible, with a minimum titer of *c.* 10^8 PFU/mL necessary to locate phage on the surface of a carbon-coated copper grid. Phage suspensions may be concentrated using standard molecular protocols such as ultrafiltration membranes, polyethylene glycol (PEG) precipitation, or cesium chloride equilibrium gradients (Sambrook and Russell, 2001). Simple preparations for examining *Campylobacter* phage under TEM are highlighted by Atterbury et al. (2003a), and Owens et al. (2012). Some *Campylobacter* phages have been found to be members of the *Siphoviridae* family, containing DNA inside an icosahedral head, with a long non-contractile tail. However, the majority of described *Campylobacter* phages belong to the *Myoviridae* family: possessing an icosahedral head, long contractile tail and double stranded DNA. Their average head diameters range from 80 to 140 nm, and tail lengths range from 95 to 120 nm. Interestingly, a few TEM images of *Campylobacter* phages show them in a contracted-tail state bound to spherical bodies (Loc Carrillo et al., 2007; Hwang et al., 2009; Kropinski et al., 2011), speculated to be membrane vesicles shed by *C. jejuni* as a defense mechanism against phage attack (Loeb, 1974; Kuehn and Kesty, 2005), as displayed by Fig. 1.

3.2.3. Protein profiling

Only two protein profiles of *Campylobacter* phage have been published to-date (Timms et al., 2010; Hammerl et al., 2011). Timms et al. (2010) used precast gels. They loaded their purified phage suspension (*c.* 10^{10} PFU/mL) to gradient gels using the manufacturer's SDS sample and gel running buffers, and followed the manufacturers' instructions. The gels were run at 200 V for 35–50 min, and stained with colloidal coomassie blue. Protein bands were excised and digested with trypsin, before undergoing electrospray ionization followed by tandem mass spectrometry (MS/MS). Both research groups used the Mascot Deamon web server database to analyze their peptide sequences.

3.2.4. Genomic characterization

Pulsed-Field Gel Electrophoresis has been used extensively to determine the genome size of most *Campylobacter* phages characterized to-date (Sails et al., 1998; Atterbury et al., 2003a). It is recommended to use at least a 10^9 PFU/mL suspension of phage for genome size determination, and higher titers for restriction endonuclease analysis. Once embedded in an agarose block, the protein-based structures of the phage are degraded using proteinase K and a detergent (e.g., lauryl sarkosyl). Proteinase K is then inactivated by washing the agarose block at 55 °C and the washed block can be stored in TE buffer at 4 °C for no more than 3 months. For restriction digest analysis, a number of restriction enzymes have been used, although most have failed to produce a restriction profile (Sails et al., 1998; Hwang et al., 2009; Owens et al., 2012). Table 1 highlights these restriction enzymes. The inability for most of these restriction enzymes to digest phage DNA has been due to methylation of phage DNA either via host methyltransferases or phage-encoded methylases (Kropinski et al.,

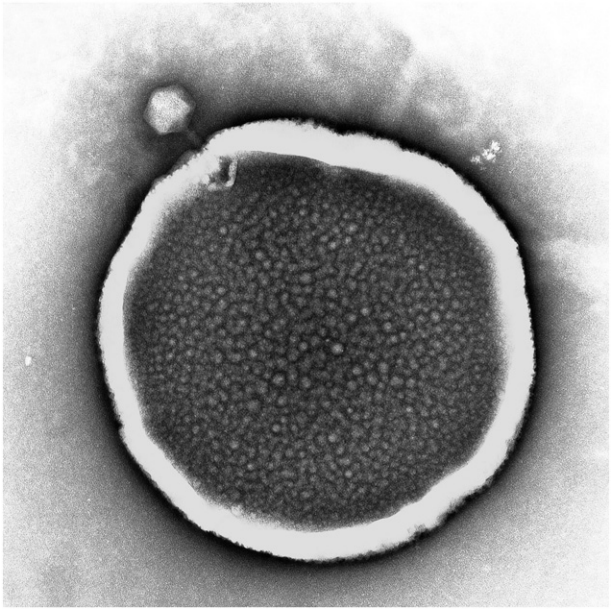

Fig. 1. TEM of *Campylobacter* phage attached to a spherical body.

Table 1
Restriction enzymes previously used to digest DNA from *Campylobacter* phages.

Unsuccessful digestions	Variability in success of digestion	Successful digestions
*Acc*I, *Alu*I, *Ava*I, *BamH*I, *Cla*I, *EcoR*I, *EcoR*V, *Hae*III, *Hind*III, *Hpa*II, *Kpn*I, *Pst*I, *Pvu*I, *Rsa*I, *Sal*I, *Sac*I, *Sca*I, *Sma*I, *Sph*I, *Ssp*I, *Xba*I, and *Xho*I	*Dra*I, *Mbo*I, *Sau3A*I, and *Taq*I	*Hha*I

Table 3
Campylobacter phage sequences published to-date.

Phage	Accession number	Reference
CP220	EMBL: FN667788	(Timms et al., 2010)
CPt10	EMBL: FN667789	(Timms et al., 2010)
NCTC12673	GenBank: NC_015464.1	(Kropinski et al., 2011)
vB_CcoM-IBB_35	Unknown	(Carvalho et al., 2012a)
CP81	GenBank: NC_019507.1	(Hammerl et al., 2012)
CP21	GenBank: NC_019507.1	(Hammerl et al., 2012)
CP30A	GenBank: NC_018861.1	—
CPX	GenBank: NC_016562.1	—

2011). Electrophoretic parameters used to analyze the PFGE profile are dependent on the speculated fragment sizes, and PFGE apparatus manufacturers such as Biorad provide helpful guidelines in their equipment manuals. As size standards the MidRange I PFG Marker by New England Biolabs or lambda concatemer have been successfully used.

Analysis at the genomic level was first performed for the set of 16 *Campylobacter* typing phages (Sails et al., 1998). The majority of *Campylobacter* phages possessing icosahedral heads and contractile tails isolated so far may be grouped according to their genome sizes as shown in Table 2 (Sails et al., 1998; Connerton et al., 2011). Coward and colleagues identified subcategories within group III by studying the development of spontaneous resistance between these phages and certain *Campylobacter* phage-type strains, although phages within groups I and II could not be subdivided. Interestingly, resistance to phages in groups I and II have been associated with motility defects, whereas resistance to phages in group III have been associated with disruption of capsular polysaccharide (CPS) expression (Coward et al., 2006).

Preparation of genomic DNA for sequencing requires highly purified, high titer phage suspensions. A variety of protocols have been used to isolated *Campylobacter* phage DNA, as has the technology to generate DNA libraries, including use of the Roche's 454 Genome Sequencer FLX system (Timms et al., 2010; Hammerl et al., 2011; Carvalho et al.,2012b); proprietary technology by Fidelity Systems (Kropinski et al., 2011); and shotgun sequencing (Timms et al., 2010). The extraction methods used to obtain *Campylobacter* phage DNA plays an important part in the information obtained. Proteins, which have been found to be tightly bound to phage DNA, tend to be removed during the phenol extraction procedure along with a significant amount of (>80%) DNA. Separation of DNA from proteins has also proved problematic with the Qiagen genomic DNA purification kit columns (Kropinski et al., 2011). To add, phage genome sequencing has proved difficult due to their refractory properties to restriction enzyme digestion and an inability to amplify DNA with regular *Taq* polymerase. It may be worth noting that due to the small number of phage genomes sequenced so far, and the limited bioinformatics data available to identify and annotate genes, sequencing a phage does not guarantee to determine if the phage carries 'unknown' genes coding for lysogeny, or ones that can promote a bacterium's virulence or resistance properties (Carvalho et al., 2012b). To date, six *Campylobacter* phage sequences have been published, as highlighted in Table 3.

3.2.5. Campylobacter prophages

The ability for bacteria to adapt and survive in various environments is dependent on their genetic diversity. In addition to virulent phages, temperate phages tend to be important for the genetic evolution and virulence of *Campylobacters*. The genetic diversity and instability of *C. jejuni* strains have been shown to be due in part to the translocation of a Mu-like prophage sequence that was first discovered in the genome of *C. jejuni* strain RM1221, which was also inducible by mitomycin C (Fouts et al., 2005). Although initial attempts at inducing temperate phage from *Campylobacter* were unsuccessful (Grajewski et al., 1985; Salama et al., 1989), subsequent to the genome sequence of *C. jejuni* RM 1221, prophages have been found to be widely distributed in 52 of 365 *Campylobacter* isolates tested for the presence of the Mu-like prophage (Clark, 2011). The presences of homologues sequences to this prophage have been found to affect molecular typing results such as PFGE patterns (Barton et al., 2007). Detailed analysis of *Campylobacter* prophage sequences have revealed low GC content and repeats often encountered in their host genome (Clark and Ng, 2008). Southern blots and PCR-based techniques were employed to search for prophage sequences found in *C. jejuni* strain RM1221. The majority of analyses have been performed at the sequence level because common prophage induction techniques (such as use of mitomycin C) do not work well with *Campylobacter* species.

Table 2
Taxanomic grouping of *Campylobacter* phages based on genome size and phage resistance development.

Group	Average genome size (Kb)	Phage resistance associated with	Frequency of isolation
I	320	Motility defect	Rare
II	185	Motility defect	Uncommon
III	135	Capsular polysaccharide structure	Very frequently isolated

3.2.6. Interaction between Campylobacter phages and their hosts

Since phage genome sequencing is still in its infancy stage, it is important to study the phage-host interactions through both *in-vitro* and *in-vivo* studies in order to understand development of resistance. Holst Sorensen et al. (2011) used the well-characterized *Campylobacter jejuni* NCTC11168 strain to select for a phage-resistant mutant, in order to determine the receptor site for *Campylobacter* phage F336. By using periodate or proteinase K, they were able to elucidate that phage binding was due to a carbohydrate moiety rather than an outer membrane protein on the surface of the phage-resistant mutant strain. With the aid of high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) analysis of intact bacterial cells, they found that the difference between the susceptible wild type and resistant strain was a modification of the capsular polysaccharide's (CPS) hypervariable *O*-methyl phosphoramidate structure. This structure has been found to be highly abundant in *C. jejuni* strains although it is unknown how a defect within this structure affects the virulence of the resistant-mutant strain (Holst Sorensen et al., 2011). Scott et al. (2007a) used *in-vivo* studies to investigate how *Campylobacter* phage CP34 predation upon *C. jejuni* HPC5 populations in the avian gut, would affect the development of phage-resistant mutants. Three phenotypes were discovered from the strains isolated: resistance to phage, poor colonisers of the chicken intestine, and producers of a functional Mu-like phage, although these were not seen with *in-vitro* kinetics studies. These phenotypes resulted from intra-genomic inversions between Mu-like prophage sequences present in the parental strain. Interestingly, the reintroduction of these phenotypes to chikens in the adsence of phage predation resulted in bacterial genomic rearrangements that lead the population to regain their competency at colonizing the chicken gut as well as revert back to being sensitive to phage (Scott et al., 2007a).

The intriguing differences between results obtain from *in-vitro* and *in-vivo* kinetic studies of phage-host interactions can be highlighted by the work published by Loc Carrillo et al. (2005). A particular *C. jejuni* strain (HPC5) was tested for its development of phage resistance to two

different *Campylobacter* phages (CP8 and CP34) both *in-vitro* and *in-vivo*. *In-vitro* studies showed 8% and 11% of the colonies tested became resistant to successive CP8 and CP34 phage infections, respectively. In contrast, only 4% of colonies recovered from the CP34 phage treated *in-vivo* studies gained resistance. Furthermore, isolates from the *in-vitro* studies were not found to revert back to the sensitive phenotype after five consecutive subcultures. Kinetic differences between these two phages and the target bacteria were also found to have considerably different behaviors between their *in-vitro* and *in-vivo* environments, as highlighted below (Loc Carrillo et al., 2005).

4. Use of phages to control *Campylobacter* in live birds and food products

To-date, there have been a few studies looking at the feasibility to use *Campylobacter* phages to control these pathogenic bacteria both at the farm-level and on the processed carcass. Once a phage has been characterized and deemed suitable for applications as an antibacterial agent, there will be a number of *in-vivo* experiments necessary to turn theory into practice.

4.1. Use in live birds

Table 4 highlights some *in-vivo* studies, which have investigated the potential of phages to prevent and/or control *Campylobacter* in the intestine of experimentally colonized birds. Wagenaar and colleagues were one of the first to investigate the application of a single *Campylobacter* phage type in live broilers as well as test the effect of a two-phage treatment. They postulated that the addition of a second phage might reduce the rate of phage-resistant mutants developing, although the presence of such mutants was not tested for. The authors state that the efficacy of the combination of phages was comparable to their individual effects seen with *in-vitro* studies, to determine that no antagonistic effect would occur (Wagenaar et al., 2005).

Loc-Carrillo and colleagues studied two *Campylobacter* phages (CP8 and CP34) in an *in-vivo* model, based on *in-vitro* efficacy studies. Birds pre-colonized with two different wild-type *Campylobacter* strains resulted in different outcomes after being treated with phage CP8. A significant drop in the population of *C. jejuni* GIIC8 colonizing the ceca, after 1 day of treatment, was seen compared to the relatively unaffected HPC5 population. However, when phage CP34 was used, the treatment produced different results to phage CP8 against the *C. jejuni* HPC5 colonizing the ceca, where the drop in bacterial load was not only lower but also maintained for up to day 5 post treatment. Interestingly, the *in-vivo* results were contrary to *in-vitro* results and the ability of phage CP8 in killing *C. jejuni* HPC5 *in-vitro* was not reproduced in the treated chickens (Loc Carrillo et al., 2005), most likely due to the vastly different environments the bacterial population encountered between the chicken gut and as homogenously grown planktonic cells in a flask.

El-Shibiny et al. (2009) conducted similar *in-vivo* studies with a wild-type *C. coli* OR12 strain that was used to colonize the birds and treated with the same phage dosages. Only the highest dose of 10^9 PFU reduced the *Campylobacter* counts in the ceca by 1–2 Logs within the first 2 days (El-Shibiny et al., 2009).

Carvalho and colleagues tested the efficacy of a phage cocktail on chicks colonized by *C. jejuni* or *C. coli*. The phages chosen had different complimentary lytic spectra. Phage treatment delivered through the feed appeared to produce a slightly higher reduction of *C. coli* when compared to administration by oral gavage. To minimize the number of birds culled over the whole study, fecal and cloacal swab samples were used to enumerate the number of *Campylobacter* excreted by the chicks. Investigating the rate of phage resistance development in *Campylobacters* passaged through the chick guts, they found 6% of the isolates were resistant to phage despite not being exposed to

Table 4
In−vivo phage application to control *Campylobacter* in birds.

Type of phage application	*Campylobacter* phage(s) used	Treatment plan	Outcome	Reference
Preventative	Phage 71 (NCTC12671)	1. Single (10^{10} PFU) dose given to 7-day old chicks 2. Chicks challenged with *C. jejuni* on day 10 3. Daily (10^{10} PFU) dose proceeds until day 16 4. Birds monitored up to day 42	* Delayed on-set of colonization * 1 Log lower cecal content	Wagenaar et al., 2005
Therapeutic	Phage 71 (NCTC12671)	1. Chicks challenged with *C. jejuni* on day 10 2. Daily (10^{10} PFU) dose given from day 15 until day 20 3. Birds monitored up to day 42	* 3 Log reduction in the first 48 h * By end of treatment cecal content matched results to preventative group	Wagenaar et al., 2005
Therapeutic	Cocktail of two phages: 71 and 69	1. Birds challenged with *C. jejuni* on day 32 2. Daily (10^{10} PFU) dose given on days 39 to 42 3. Birds monitored up to day 58	* 1.5 Log reduction seen during treatment period * Steady increase then stabilization after 5 days * 1 Log lower cecal content compared to untreated group	Wagenaar et al., 2005
Therapeutic	Phage CP8	1. Birds challenged with *C. jejuni* on day 20 2. Three (10^5, 10^7, and 10^9 PFU) doses of phage tested on day 25 3. Birds monitored up to day 30	When birds colonized with HPC5: * Phage persisted in intestine over the 5 days * 1 Log reduction achieved on first day * No difference in cecal content compared to untreated group When birds colonized with GII8: • Substantial reduction seen in first 3 days	Loc Carrillo et al., 2005
Therapeutic	Phage CP34	1. Birds challenged with *C. jejuni* on day 20 2. Three (10^5, 10^7, and 10^9 PFU) doses of phage tested on day 25 3. Birds monitored up to day 30	* The 10^7 PFU dose produced largest reduction	Loc Carrillo et al., 2005
Therapeutic	Phage CP220	1. Birds challenged with *Campylobacter* on day 20 2. Three (10^5, 10^7, and 10^9 PFU) doses of phage tested on day 25 3. Birds monitored up to day 30	When birds colonized with *C. jejuni:* * 10^7 PFU dose produced 2 Log reduction in first 2 days When birds colonized with *C. coli:* * 10^9 PFU produced 1–2 Logs in first 2 days	El-Shibiny et al. (2009)
Therapeutic	Cocktail of 3 phages: phiCcoIBB35 phiCcoIBB37 phiCcoIBB12	1. Chicks challenged with *C. jejuni* on day 1 2a. Single 10^6 PFU dose administered on day 7 by oral gavage 2b. Phage feed given as 10^7 PFU in 45 g chick crumbs	* 2 Log reduction seen up to 7 days post-treatment with oral gavage * 0.5 Log higher reduction seen with feed compared to oral gavage	Carvalho et al. (2010b)

the phage treatment, in comparison to 13% of isolates that showed phage resistance after being exposed to phage treatment. After passaging the resistant strains back through the chicken gut, they found 86% of the colonies that were previously phage-resistant without exposure to phage had reverted back to being sensitive, whereas only 54% of the colonies that were phage-resistant after being exposed to the phage treatment reverted back to their sensitive phenotype (Carvalho et al., 2010b).

To examined the success of one *C. jejuni* genotype over another under the influence of phage predation *in-vivo*, Scott and colleagues used three different wild-type *Campylobacter* strains to colonize chicken guts, singly (i.e., either with F2E1$^{\phi R}$ or F2E3$^{\phi S}$ or F2C10$^{\phi S}$ strains) or in paired competition (i.e., F2E3$^{\phi S}$ and F2C10$^{\phi S}$ strains) with and without *Campylobacter* phage CP30 to determine any colonization benefits or cost associated to a phage-resistant phenotype. As expected, under a phage predation environment, colonization levels of the phage-resistant strain did not differ to those found in the absence of phage-treatment, whereas reduced levels in the populations of the phage sensitive strains were seen with the phage-treatment. *In-vivo* studies of competitive colonization without a phage predation pressure demonstrated that the phage-sensitive strains could out-compete the phage-resistant strain. However, in the presence of phage-treatment, the situation was reversed and the phage-resistant strain was able to out-compete both phage-sensitive strains. The authors concluded that phage predation can influence *Campylobacter* populations that colonize chickens, selecting for particular variants between these populations. For the competitive colonization studies, the group used neomycin in their CCDA media to differential between F2E1$^{\phi R}$ and F2E3$^{\phi S}$ isolates, as well as colony blot and hybridization DIG-labeled probes specific for F2E1$^{\phi R}$ and F2E3/F2C10$^{\phi S}$ to differentiate F2E1$^{\phi R}$ isolates from F2E3$^{\phi S}$ or F2C10$^{\phi S}$ isolates (Scott et al., 2007b).

In a later study, Sørensen et al. (2012) found that the phase variable *O*-methyl phosphoramidate (MeOPN) moiety of the *C. jejuni* capsular polysaccharide (CPS) is the receptor involved in hindering phage susceptibility of resistant mutants isolated after the co-infection of chickens with both *C. jejuni* NCTC 11168 and phage F336. Most interestingly, these mutants also gained resistance to four other phages. The investigators postulate that the constant exposure of *Campylobacter* to phage predation in the chicken gut selects for different phage-host co-evolution (Sørensen et al., 2012).

From the six *in-vivo* studies mentioned above, it may be worth considering the criteria outlined in the checklist provided, when setting-up future phage application studies (Box 1). Parameters that have been investigated include a range of dosages, which have surprisingly shown that the highest phage titer does not necessarily result in the biggest reduction when compared to lower titers (Loc Carrillo et al., 2005). In addition, results from *in-vitro* studies, although insightful, cannot be used to predict how the application of phages as antibacterial agents will work *in-vivo*. Multiple dosing of phage-treatment can help suppress bacterial counts in feces during treatment; however, cessation of treatment results in a rapid increase in bacterial load, although still lower than the untreated controls (Wagenaar et al., 2005). Timing of treatment is crucial, as most phages studied so far have shown efficacy at reducing bacterial loads (up to 3 Logs) within the first 2–3 days; however, regeneration of the *Campylobacter* population steadily occurs after this time. Oral gavage has been the main route for administering phages, although foods such as chick crumbs have been used (Carvalho et al., 2010b), which appears to be more practical if phage treatment is to be consider feasible by the poultry industry. The delivery of phages through the birds' drinking water has also been contemplated, although this has not yet been tested. Phage cocktails have also been investigated (Wagenaar et al., 2005; Carvalho et al., 2010b) in order to account for the heterogeneous nature of *Campylobacter*, although no significant improvement in their efficacy was seen when compared to birds treated with individual phages. With that said, it is apparent that the use of phage cocktails will target a broader range of bacteria, than treatments containing only single phages.

Box 1
Checklist for in-vivo studies.

- ☐ Determine appropriate host(s) susceptible to phage(s), capable of supporting an appropriate and reproducible in-vivo colonization model.
- ☐ Use 4–5 birds per time point to enumerate bacterial load.
- ☐ Use birds that are appropriately aged to reflect the practical applications.
- ☐ Administer phage with 30% w/v $CaCO_3$ to compensate for acidic levels in chicken gut for oral gavages, or mix phage with food.
- ☐ If using multiple Campylobacter strains, use appropriate methods for isolating and differentiating between isolates.
- ☐ Test at least 10% of the colonies recovered from phage-treated birds to determine rate of resistance.

4.2. Use in food products

The use of phages to control the level of *Campylobacter* contamination on the surface of chicken carcasses has also been investigated by a number of research groups. Table 5 highlights the outcomes from three studies. Two of the studies used chicken skin tainted with know concentrations of susceptible *Campylobacter* cells (Goode et al., 2003; Atterbury et al., 2003b), while another used raw and cooked beef products with high and low bacterial loads treated with high and low phage titers (Bigwood et al., 2008). In a later study involving polynomial modeling, Bigwood and colleagues calculated that an MOI of 10^5 would be required to kill at least 25% of a susceptible bacterial population on the surface of food within a 2 h period (Bigwood et al., 2009).

5. Challenges and further research

It is clear that much work is still required to understand how best to use phages to control pathogenic bacteria, although some progression has been made by the *in-vivo* and *in-situ* studies mentioned above. *In-vitro* studies are important at helping to understand the kinetics of phages against target hosts although they cannot be solely relied upon since investigators have shown that there are discrepancies between *in-vitro* and *in-vivo* results (Loc Carrillo et al., 2005).

On a laboratory scale, conventional methods such as liquid culture or plate lysis methods can be employed for propagating *Campylobacter* phages in small to medium amounts (i.e., 10 mL to 1000 mL). However, some of these phages are difficult to propagate in liquid culture and titers may vary considerably between batches. In addition, the plate lysis method usually produces phage titers *c.* 10^8 PFU/mL, which when considering that high phage titers are required for some studies, optimization of propagation methods will need to be developed. For larger phage stock volumes (i.e., >1 L), particularly important when conducting large *in-situ* animal trials, the ability to scale-up phage production will play an important part in considering the commercial applications of phages. On an industrial scale, where large fermentation vats are used to grow the propagation hosts, safety issues of growing large volumes of a pathogen would be of great concern, and attenuated strains capable of producing high phage titers would therefore be needed.

To-date, all phage treatment studies against *Campylobacter* have involved using filtered phage lysates with no report of any adverse effects seen from the recipients (i.e. chickens); however, the use of highly purified phage preparations will need to be considered when

Table 5
In-situ phage application to control *Campylobacter* in food products.

Type of phage application	*Campylobacter* phage(s) used	Treatment plan	Outcome	Reference
Food decontamination	Phage NCTC 12673	1. Phage applied (10^6 PFU/cm^2) to tainted chicken skin and stored at 4 °C for 24 h	* 1 Log reduction seen with treated compared to untreated group	Goode et al., 2003
Food decontamination	Phage NCTC 12674	1. Phage applied to chicken skin (from a range between 10^{4-7} PFU) 2. Skin stored at −20 °C or 4 °C for up to 5 days	* 2 Log drop recovered from frozen-thawed samples * 1 Log drop seen from fresh samples *No phage amplification detected *No phage-resistant mutants recovered	Atterbury et al., 2003b
Food decontamination	Phage Cj6	1. Phage applied to raw and cooked contaminated beef products 2. Skin stored at 5 °C or 24 °C for up to 8 days	* 2 Log reduction seen with meats containing high densities of host cells * Similar results seen at both storage temperatures	Bigwood et al., 2008
Food decontamination	Phage: NCTC12684, or CP81	1. Phage applied to raw chicken meat at MOIs of 10 or 100 2. Meat stored at 4 °C for up to 7 days	* No reduction in bacterial load was seen at 4 °C	Orquera et al., 2012

trying to apply phages to ready-to-eat foods, since the presence of bacterial toxins would have a detrimental effect on the consumer of those phage treated products. One of the latest techniques used to purify and concentrate phage preparations involves convective interaction media (CIM) monolithic columns, which have been previously been used to purify and concentrate phages like phage T4 (Smrekar et al., 2008). With further optimization, this technology may be used in the future to purify large volumes of phage preparations in order to remove endotoxins routinely produced by Gram-negative bacteria such as *Campylobacter*.

Another hurdle in the application of phages to poultry, and in particular live birds at the farm, is the development of phage-resistant mutants (Loc Carrillo et al., 2005; Coward et al., 2006; Scott et al., 2007a; Carvalho et al., 2010b). Further research is still needed to answer which type of resistance is actually relevant for phage therapy and other phage applications. This is particularly the case when considering the use of phages in the primary production setting such as in chick hatcheries (Carvalho et al., 2012b). There is no current data available on the distribution of phage resistant strains on a farm environment following phage treatment of birds. It will also be interestingly to see a phage therapy trial on naturally colonized broilers within a farm environment.

The development of phage resistant mutants is thought to be less unlikely when applying to commercial birds 1–2 days before going through the processing plant. The prevalence of phage resistant mutants may also be very limited when applying phages to carcasses or food products stored at 4 °C, as studies have shown no bacterial proliferation occurring (Atterbury et al., 2003b; Goode et al., 2003). The next step would be to convince consumers that there are such things as 'good' viruses!

6. Concluding remarks

The annual estimated cases of campylobacteriosis worldwide is 400–500 million, and the transmission of *Campylobacter* to humans is attributed to the consumption of contaminated foods of animal origins particularly poultry meat (Luangtongkum et al., 2009). Reducing the load of *Campylobacter* present in poultry at the farm and on the carcass can save millions from the cost incurred by the loss of productivity by the sufferer as well as from cases that lead to hospital treatments. The use of phages to control the levels of *Campylobacter* present in chickens has been explored since the start of the millennium, and it is a particularly appealing method against antibiotic-resistant strains, which have been steadily on the rise since the 1990s.

The use of phages to control pathogenic bacteria in the food industry is an attractive concept since not only are they abundant in nature (Hendrix et al., 1999) but they have also been found widely present on foods (Whitman and Marshall, 1971; Atterbury et al., 2003a) as well as isolated from the human intestine (Reyes et al., 2010). Recently, Goodridge and Bisha (2011) reviewed the potential use of phages to control other bacterial pathogens present in animals used for food production and in food products themselves, and concluded that research studies have demonstrated the usefulness of phage application in those settings (Goodridge and Bisha, 2011). It is clear that phage research and looking at their applications to control bacteria has gained considerable interest in the past decade particularly since a number of commercial phage-based products are now available to apply on animal products and ready-to-eat foods (Monk et al., 2010). Phage-based products against *Campylobacter* have not yet reached the market place but a few companies have publically declared interest in developing such products including Intralytix, GangaGen and Micreos.

References

Ackermann, H.W., 2007. 5500 Phages examined in the electron microscope. Arch. Virol. 152, 227–243.

Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D., Connerton, I.F., 2003a. Isolation and characterization of campylobacter bacteriophages from retail poultry. Appl. Environ. Microbiol. 69, 4511–4518.

Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D., Connerton, I.F., 2003b. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. Appl. Environ. Microbiol. 69, 6302–6306.

Atterbury, R.J., Dillon, E., Swift, C., Connerton, P.L., Frost, J.A., Dodd, C.E.R., Rees, C.E.D., Connerton, I.F., 2005. Correlation of Campylobacter bacteriophage with reduced presence of hosts in broiler chicken ceca. Appl. Environ. Microbiol. 71 (8), 4885–4887.

Barton, C., Ng, L.K., Tyler, S.D., Clark, C.G., 2007. Temperate bacteriophages affect pulsed-field gel electrophoresis patterns of Campylobacter jejuni. J. Clin. Microbiol. 45 (2), 386–391.

Bigwood, T., Hudson, J.A., 2009. Campylobacters and bacteriophages in the surface waters of Canterbury (New Zealand). Lett. Appl. Microbiol. 48, 343–348.

Bigwood, T., Hudson, J.A., Billington, C., Carey-Smith, G.V., Heinemann, J.A., 2008. Phage inactivation of foodborne pathogens on cooked and raw meat. Food Microbiol. 25, 400–406.

Bigwood, T., Hudson, J.A., Billington, C., 2009. Influence of host and bacteriophage concentrations on the inactivation of food-borne pathogenic bacteria by two phages. FEMS Microbiol. Lett. 291, 59–64.

Butzler, J.P., 2004. Campylobacter, from obscurity to celebrity. Clin. Microbiol. Infect. 10, 868–876.

Canchaya, C., Fournous, G., Chibani-Chennoufi, S., Dillmann, M.-L., Brüssow, H., 2003. Phage as agents of lateral gene transfer. Curr. Opin. Microbiol. 6, 417–424.

Carvalho, C., Susano, M., Fernandes, E., Santos, S., Gannon, B., Nicolau, A., Gibbs, P., Teixeira, P., Azeredo, J., 2010a. Method for bacteriophage isolation againt target Campylobacter strains. Lett. Appl. Microbiol. 50, 192–197.

Carvalho, C.M., Gannon, B.W., Halfhide, D.E., Santos, S.B., Hayes, C.M., Roe, J.M., Azeredo, J., 2010b. The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and Campylobacter jejuni in chickens. BMC Microbiol. 10, 232.

Carvalho, C.M., Kropinski, A.M., Lingohr, E.J., Santos, S.B., King, J., Azeredo, J., 2012a. The genome and proteome of a Campylobacter coli bacteriophage vB_CcoM-IBB_35 reveal unusual features. Virol. J. 9, 35.

Carvalho, C.M., Santos, S.B., Kropinski, A.M., Ferreira, E.C., Azeredo, J., 2012b. Phages as therapeutic tools to control major foodborne pathogens: Campylobacter and Salmonella. 179–214.

Clark, C.G., 2011. Sequencing of CJIE1 prophages from Campylobacter jejuni isolates reveals the presence of inserted and (or) deleted genes. Can. J. Microbiol. 57 (10), 795–808.
Clark, C.G., Ng, L.-K., 2008. Sequence variability of Campylobacter bacteriophages. BMC Microbiol. 8, 49.
Coker, A.O., Isokpehi, R.D., Thomas, B.N., Amisu, K.O., Obi, C.L., 2002. Human campylobacteriosis in developing countries. Emerg. Infect. Dis. 8, 237–244.
Connerton, P.L., Loc Carrillo, C.M., Swift, C., Dillon, E., Scott, A., Rees, C.E.D., Dodd, C.E.R., Frost, J., Connerton, I.F., 2004. Longitudinal study of Campylobacter jejuni bacteriophages and their hosts from broiler chickens. Appl. Environ. Microbiol. 70, 3877–3883.
Connerton, P.L., Timms, A.R., Connerton, I.F., 2011. Campylobacter bacteriophages and bacteriophage therapy. J. Appl. Microbiol. 111, 255–265.
Coward, C., Grant, A.J., Swift, C., Philp, J., Towler, R., Heydarian, M., Frost, J.A., Maskell, D.J., 2006. Phase-variable surface structures are required for infection of Campylobacter jejuni by bacteriophages. Appl. Environ. Microbiol. 72, 4638–4647.
E.F.S.A., 2011. Scientific opinion on campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J. 9, 2105.
El-Shibiny, A., Connerton, P.L., Connerton, I.F., 2005. Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens. Appl. Environ. Microbiol. 71, 1259–1266.
El-Shibiny, A., Scott, A., Timms, A., Metawea, Y., Connerton, P., Connerton, I., 2009. Application of a group II Campylobacter bacteriophage to reduce strains of Campylobacter jejuni and Campylobacter coli colonizing broiler chickens. J. Food Prot. 72, 733–740.
Fouts, D.E., Mongodin, E.F., Mandrell, R.E., Miller, W.G., Rasko, D.A., Ravel, J., Brinkac, L.M., DeBoy, R.T., Parker, C.T., Daugherty, S.C., Dodson, R.J., Durkin, A.S., Madupu, R., Sullivan, S.A., Shetty, J.U., Ayodeji, M.A., Shvartsbeyn, A., Schatz, M.C., Badger, J.H., Fraser, C.M., Nelson, K.E., 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. PLoS Biol. 3, e15.
Frost, J.A., Kramer, J.M., Gillanders, S.A., 1999. Phage typing of Campylobacter jejuni and Campylobacter coli and its use as an adjunct to serotyping. Epidemiol. Infect. 123, 47–55.
Goode, D., Allen, V.M., Barrow, P.A., 2003. Reduction of experimental salmonella and campylobacter contamination of chicken skin by application of lytic bacteriophages. Appl. Environ. Microbiol. 69, 5032–5036.
Goodridge, L.D., Bisha, B., 2011. Phage-based biocontrol strategies to reduce foodborne pathogens in foods. Bacteriophage 1, 130–137.
Grajewski, B.A., Kusek, J.W., Gelfand, H.M., 1985. Development of a bacteriophage typing system for Campylobacter jejuni and Campylobacter coli. J. Clin. Microbiol. 22, 13–18.
Guerin, M.T., Sir, C., Sargeant, J.M., Waddell, L., O'Connor, A.M., Wills, R.W., Bailey, R.H., Byrd, J.A., 2010. The change in prevalence of Campylobacter on chicken carcasses during processing: a systematic review. Poult. Sci. 89, 1070–1084.
Hale, C.R., Scallan, E., Cronquist, A.B., Dunn, J., Smith, K., Robinson, T., Lathrop, S., Tobin-D'Angelo, M., Cloghen, P., 2012. Estimates of enteric illness attributable to contact with animals and their environments in the United States. Clin. Infect. Dis. 54 (Suppl. 5), S472–S479.
Hammerl, J.A., Jackel, C., Reetz, J., Beck, S., Alter, T., Lurz, R., Barretto, C., Brussow, H., Hertwig, S., 2011. Campylobacter jejuni group III phage CP81 contains many T4-like genes without belonging to the T4-type phage group: implications for the evolution of T4 phages. J. Virol. 85, 8597–8605.
Hammerl, J.A., Jackel, C., Reetz, J., Hertwig, S., 2012. The complete genome sequence of bacteriophage CP21 reveals modular shuffling in Campylobacter group II phages. J. Virol. 86, 8896.
Hansen, V.M., Rosenquist, H., Baggesen, D.L., Brown, S., Christensen, B.B., 2007. Characterization of Campylobacter phages including analysis of host range by selected Campylobacter Penner serotypes. BMC Microbiol. 7, 90.
Hendrix, R.W., Smith, M.C., Burns, R.N., Ford, M.E., Hatfull, G.F., 1999. Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage. Proc. Natl. Acad. Sci. U. S. A. 96, 2192–2197.
Hoffmann, S., Batz, M.B., Morris Jr., J.G., 2012. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J. Food Prot. 75, 1292–1302.
Holst Sorensen, M.C., van Alphen, L.B., Harboe, A., Li, J., Christensen, B.B., Szymanski, C.M., Brondsted, L., 2011. Bacteriophage F336 recognizes the capsular phosphoramidate modification of Campylobacter jejuni NCTC11168. J. Bacteriol. 193, 6742–6749.
Hwang, S., Yun, J., Kim, K.P., Heu, S., Lee, S., Ryu, S., 2009. Isolation and characterization of bacteriophages specific for Campylobacter jejuni. Microbiol. Immunol. 53, 559–566.
Kropinski, A.M., Arutyunov, D., Foss, M., Cunningham, A., Ding, W., Singh, A., Pavlov, A.R., Henry, M., Evoy, S., Kelly, J., Szymanski, C.M., 2011. Genome and proteome of Campylobacter jejuni bacteriophage NCTC 12673. Appl. Environ. Microbiol. 77, 8265–8271.
Kuehn, M.J., Kesty, N.C., 2005. Bacterial outer membrane vesicles and the host-pathogen interaction. Genes Dev. 19, 2645–2655.
Kutter, E., 2009. Phage host range and efficiency of plating. Methods Mol. Biol. 501, 141–149.
Kuznetsov, Y.G., Chang, S.C., McPherson, A., 2011. Investigation of bacteriophage T4 by atomic force microscopy. Bacteriophage 1, 165–173.
Lindberg, A.A., 1973. Bacteriophage receptors. Annu. Rev. Microbiol. 27, 205–241.
Loc Carrillo, C., 2005. Bacteriophage control of campylobacters in poultry production. PhD Thesis Univerity of Nottingham.
Loc Carrillo, C., Atterbury, R.J., El-Shibiny, A., Connerton, P.L., Dillon, E., Scott, A., Connerton, I.F., 2005. Bacteriophage therapy to reduce Campylobacter jejuni colonization of broiler chickens. Appl. Environ. Microbiol. 71, 6554–6563.
Loc Carrillo, C.M., Connerton, P.L., Pearson, T., Connerton, I.F., 2007. Free-range layer chickens as a source of Campylobacter bacteriophage. Antonie Van Leeuwenhoek 92, 275–284.
Loeb, M.R., 1974. Bacteriophage T4-mediated release of envelope components from *Escherichia coli*. J. Virol. 13, 631–641.
Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C.M., Zhang, Q., 2009. Antibiotic resistance in Campylobacter: emergence, transmission and persistence. Future Microbiol. 4, 189–200.
Man, S.M., 2011. The clinical importance of emerging Campylobacter species. Nat. Rev. Gastroenterol. Hepatol. 8, 669–685.
Meredith, H., McDowell, D., Bolton, D.J., 2013. An evaluation of trisodium phosphate, citric acid and lactic acid cloacal wash treatments to reduce Campylobacter, total viable counts (TVC) and total enterobacteriaceae counts (TEC) on broiler carcasses during processing. Food Control 32, 149–152.
Monk, A.B., Rees, C.D., Barrow, P., Hagens, S., Harper, D.R., 2010. Bacteriophage applications: where are we now? Lett. Appl. Microbiol. 51, 363–369.
Moore, J.E., Corcoran, D., Dooley, J.S., Fanning, S., Lucey, B., Matsuda, M., McDowell, D.A., Megraud, F., Millar, C., O'Mahony, R., O'Riordan, L., O'Rurke, M., Rao, J.R., Rooney, P.J., Sails, A., Whyte, P., 2005. Campylobacter. Vet. Res. 36, 351–382.
Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on Campylobacter in broiler meat. Int. J. Food Microbiol. 129, 107–123.
Newell, D.G., Fearnley, C., 2003. Sources of campylobacter colonization in broiler chickens. Appl. Environ. Microbiol. 69, 4343–4351.
Newell, D.G., Elvers, K.T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N.J., Davies, R., Connerton, I., Pearson, D., Salvat, G., Allen, V.M., 2011. Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Appl. Environ. Microbiol. 77, 8605–8614.
Orquera, S., Gölz, G., Hertwig, S., Hammerl, J., Sparborth, D., Joldic, A., Alter, T., 2012. Control of Campylobacter spp. and Yersinia enterocolitica by virulent bacteriophages. Journal of Molecular and Genetic Medicine: An International Journal of Biomedical Research, 6, 273.
Owens, J., Barton, M.D., Heuzenroeder, M.W., 2012. The isolation and characterization of Campylobacter jejuni bacteriophages from free range and indoor poultry. Vet. Microbiol. 162 (1), 144–150.
Payne, R.J., Jansen, V.A., 2003. Pharmacokinetic principles of bacteriophage therapy. Clin. Pharmacokinet. 42, 315–325.
Reyes, A., Haynes, M., Hanson, N., Angly, F.E., Heath, A.C., Rohwer, F., Gordon, J.I., 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466, 334–338.
Riedel, C.T., Brndsted, L., Rosenquist, H., Haxgart, S.N., Christensen, B.B., 2009. Chemical decontamination of *Campylobacter jejuni* on chicken skin and meat. J. Food Prot. 72, 1173–1180.
Sails, A.D., Wareing, D.R., Bolton, F.J., Fox, A.J., Curry, A., 1998. Characterisation of 16 Campylobacter jejuni and C. coli typing bacteriophages. J. Med. Microbiol. 47, 123–128.
Salama, S.F., Bolton, J., Hutchinson, D.N., 1989. Improved method for the isolation of Campylobacter jejuni and Campylobacter coli bacteriophages. Lett. Appl. Microbiol. 8, 5–7.
Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual. CSHL Press.
Sampers, I., Habib, I., De Zutter, L., Dumoulin, A., Uyttendaele, M., 2010. Survival of Campylobacter spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. Int. J. Food Microbiol. 137, 147–153.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Woddowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne Illness Acquired in the United States—Major Pathogens. Emerg. Infect. Dis. 17, 7–15.
Scott, A.E., Timms, A.R., Connerton, P.L., Loc Carrillo, C., Adzfa Radzum, K., Connerton, I.F., 2007a. Genome dynamics of Campylobacter jejuni in response to bacteriophage predation. PLoS Pathog. 3, e119.
Scott, A.E., Timms, A.R., Connerton, P.L., El-Shibiny, A., Connerton, I.F., 2007b. Bacteriophage influence Campylobacter jejuni types populating broiler chickens. Environ. Microbiol. 9, 2341–2353.
Smrekar, F., Ciringer, M., Peterka, M., Podgornik, A., Štrancar, A., 2008. Purification and concentration of bacteriophage T4 using monolithic chromatographic supports. J. Chromatogr. B 861 (2), 177–180.
Sørensen, M.C.H., van Alphen, L.B., Fodor, C., Crowley, S.M., Christensen, B.B., Szymanski, C.M., Brøndsted, L., 2012. Phase variable expression of capsular polysaccharide modifications allows Campylobacter jejuni to avoid bacteriophage infection in chickens. Front. Cell. Infect. Microbiol. 2, 11.
Timms, A.R., Cambray-Young, J., Scott, A.E., Petty, N., Connerton, P.L., Clarke, L., Seeger, K., Quail, M., Cummings, N., Maskell, D.J., Thomson, N.R., Connerton, I.F., 2010. Evidence for a lineage of virulent bacteriophages that target Campylobacter. BMC Genomics 11, 214.
Wagenaar, J.A., Van Bergen, M.A., Mueller, M.A., Wassenaar, T.M., Carlton, R.M., 2005. Phage therapy reduces Campylobacter jejuni colonization in broilers. Vet. Microbiol. 109, 275–283.
Whitman, P.A., Marshall, R.T., 1971. Isolation of psychrophilic bacteriophage-host systems from refrigerated food products. Appl. Microbiol. 22, 220–223.
Whyte, P., McGill, K., Collins, J.D., 2003. An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. Food Microbiol. 20, 111–117.
Wilson, D.J., Gabriel, E., Leatherbarrow, A.J., Cheesbrough, J., Gee, S., Bolton, E., Fox, A., Fearnhead, P., Hart, C.A., Diggle, P.J., 2008. Tracing the source of campylobacteriosis. PLoS Genet. 4 (9), e1000203.