

Phase-Variable Surface Structures Are Required for Infection of *Campylobacter jejuni* by Bacteriophages

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This study characterizes the interaction between *Campylobacter jejuni* and the 16 phages used in the United Kingdom typing scheme by screening spontaneous mutants of the phage-type strains and transposon mutants of the sequenced strain NCTC 11168. We show that the 16 typing phages fall into four groups based on their patterns of activity against spontaneous mutants. Screens of transposon and defined mutants indicate that the phage-bacterium interaction for one of these groups appears to involve the capsular polysaccharide (CPS), while two of the other three groups consist of flagellotropic phages. The expression of CPS and flagella is potentially phase variable in *C. jejuni*, and the implications of these findings for typing and intervention strategies are discussed.

Campylobacter jejuni is a major cause of food-borne gastrointestinal disease in the developed world, with an estimated one in every hundred individuals in both the United States and the United Kingdom developing *Campylobacter*-related illness each year (19). This places a considerable burden on medical resources, as well as causing great stress and discomfort for the infected individual. Moreover, although most cases are self-limiting, *Campylobacter* can cause severe postinfection complications, including bacteremia and polyneuropathies such as the Guillain-Barré and Miller-Fisher syndromes. The main routes of infection are considered to be the consumption of contaminated meat (particularly poultry), the ingestion of contaminated water or unpasteurized milk and milk products, contact with pets, and travel. Moreover, a recent study suggests that there may be as-yet-undefined sources of *Campylobacter* that significantly contribute to human infection (10).

A greater appreciation of the epidemiology of *Campylobacter* spp. will require the use of genetic techniques to compare strains isolated from various sources with those causing infections in humans. For convenience, phenotypic methods such as serotyping and phage typing continue to be used to type environmental and human isolates. Sixteen *Campylobacter*-specific phages are used for the epidemiological typing of *C. jejuni* and *C. coli* in the United Kingdom (17). The original phage-typing scheme was described in the United States in 1985 using 14 phages isolated from poultry feces (22) and was later extended to incorporate 5 more phages (31). Six of the original United States phages were combined with 10 virulent phages isolated from various sources in the United Kingdom, including pig and poultry manure and sewage effluent, to form the United Kingdom scheme (46). These 16

phages, all of the *Myoviridae* family, can be subdivided into three groups according to genome size, ultrastructure, and head size (45). The DNA of these phages is refractory to digestion by a large number of restriction enzymes, and all have icosahedral heads and long contractile, nonflexible tails. To date, 92 phage types have been identified (C. Swift, unpublished observations), a phage type being defined as two or more epidemiologically unrelated *Campylobacter* isolates giving the same phage reaction pattern (17). Phage lysis as a means to type *C. jejuni* is used with a very limited understanding of the mechanisms of bacterial resistance or sensitivity to the phage.

C. jejuni colonizes the colon of the broiler chicken to extremely high numbers; chickens reaching the abattoir can carry *Campylobacter* at 10^5 to 10^9 CFU per g of cecal contents (6). Estimates of *Campylobacter* contamination found on raw chicken sold in the United Kingdom range from 40 to 80% (16). Although improvements in biosecurity help to maintain *Campylobacter*-negative flocks, once *Campylobacter* is present in a flock there is evidence for rapid horizontal transfer resulting in infection of almost all of the birds within a few days (39, 55). This, coupled with the low infectious dose of *C. jejuni* for humans (approximately 500 to 800 organisms) (7), highlights the need for effective intervention strategies within the “farm-to-fork” process to reduce the bacterial load.

Intervention strategies such as competitive exclusion with other organisms or less pathogenic *Campylobacter*, biosecurity measures, and improved husbandry have all been suggested and used with various degrees of success (50). More recently, there has been a drive to reconsider the use of phage as a means of reducing the bacterial load prior to slaughter (13, 35, 57) or during postprocessing (2, 3, 21). The use of phage as a means to control pathogens is particularly attractive considering the increasing pressure on the veterinary and medical communities to move away from antibiotic therapy. Phages have unique advantages over antibiotics in that they can specifically target a bacterial species and are both self-replicating and

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self-limiting. More than 170 phages of *Campylobacter* spp. have been reported (45).

Before phage therapy can be adopted as a means of intervention it will be important to understand phage behavior in a naturally infected flock. Connerton et al. have shown that phage and *Campylobacter* ecology in a broiler house is complex and may play a significant role in influencing which strains of *Campylobacter* enter the human food chain (13). In addition, a greater understanding of phage-host interactions and the development of phage resistance in the host will be required before phage can be used as an intervention. To begin to characterize the molecular basis of these interactions, we screened spontaneous mutants of the *Campylobacter* phage-type strains and transposon mutants of the sequenced strain *C. jejuni* NCTC 11168 for resistance to the 16 phages used in the United Kingdom typing scheme.

MATERIALS AND METHODS

Bacterial strains, phages, and growth conditions. Bacterial strains, phages, and plasmids used in the present study are listed in Table 1. *C. jejuni* was cultured at 42°C on Muller-Hinton (MH) agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% horse blood (Sigma, Poole, United Kingdom) with 5 µg of trimethoprim/ml under standard microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) in a MACS VA500 Variable Atmosphere Work Station (Don Whitley Scientific, Shipley, United Kingdom). *Escherichia coli* strain EC100D *pir-116* was grown at 37°C on Luria-Bertani (LB) agar or broth (Oxoid). *Campylobacter* motility agar was composed of 21 g/liter MH broth (Oxoid) and 0.4% technical agar #3 (Sigma). The media used for the propagation of phage and phage screening were as follows: CBHI, 37 g/liter BHI (Oxoid) supplemented with 1 mmol CaCl₂ and 1 mmol MgSO₄; CBHIO, CBHI-0.7% Select Agar (Sigma); CPTA, 15 g/liter Nutrient Broth #2 (Sigma)-0.7% Select Agar (Sigma) supplemented with 10 mmol CaCl₂ and 1 mmol MgSO₄; NZCYM, 22 g/liter NZCYM Broth (Sigma)-1.2% Select Agar (Sigma); and NZCYO, 22 g/liter NZCYM Broth (Sigma)-0.7% Select Agar (Sigma). SM buffer per liter was 5.8 g of NaCl, 2.0 g of MgSO₄ · 7H₂O, 50 ml of Tris-HCl (pH 7.4), 5.0 ml of 2% gelatin (Sigma), and 945 ml of sterile distilled water. Where necessary for selection, media were supplemented with chloramphenicol (10 µg/ml), ampicillin (100 µg/ml), or kanamycin (25 µg/ml) as appropriate. Long-term storage of bacteria was at -80°C in Microbank vials (Prolab Diagnostics, Neston, United Kingdom).

Phage propagation. Phages were propagated essentially as described previously (17). Propagating strains (Table 1) were grown as lawns for 48 h and harvested in CBHI, and the cell density adjusted to MacFarland standard no. 1 (optical density at 550 nm = 0.25). The culture was then incubated microaerophilically with shaking for 4 h, mixed with phage (~10⁹ PFU/ml) in a 1:1 ratio, and incubated standing microaerophilically for 30 min to allow the phage to adsorb. Then, 1 ml of adsorbed suspension was mixed with 5 ml of molten NZCYO tempered to 50°C and poured into a 90-mm petri dish previously prepared with 25 ml of solid NZCYM. The agar was allowed to set, and the plates were incubated uninverted overnight at 42°C in microaerophilic conditions. Subsequently, 5 ml of SM buffer was applied to the agar, followed by incubation at 4°C overnight on a rotary shaker (100 rpm). The phage-containing SM buffer was aspirated, and the plates were washed with a further 1 ml of SM buffer. The phage suspension was sterilized by filtration through 0.2-µm-pore-size filters (Sartorius, Hannover, Germany).

Selection of spontaneous *Campylobacter* mutants resistant to a typing phage. Isolation plates were prepared by applying 1 ml of phage suspension (~10⁸ PFU/ml) onto the surface of a CPTA agar plate using a sterile disposable spreader. After drying, a single colony of the *Campylobacter* type strain of interest was spread onto the surface with a sterile disposable loop and incubated at 37°C in microaerophilic conditions for 24 h. Growth from this plate was then subcultured for 24 h on a fresh plate. Five putatively resistant colonies were picked from this plate and subcultured once more to ensure single isolates were obtained. Phage sensitivity of the resultant strain was then assessed by applying phage at a routine test dilution (ca. 10⁶ to 10⁹ PFU/ml) (17) to a lawn of confluent growth of the test strain and scoring for the presence or absence of lysis after 18 h of incubation.

Phage screening of transposon mutant libraries by spot assay. *C. jejuni* NCTC 11168 random transposon mutants generated as previously described (23) were

screened against the nine typing phages lytic for NCTC 11168 in 12-well tissue culture dishes prepared with 3 ml of CPTA per well. Bacteria were grown as lawns for 48 h, harvested, and adjusted to optical density at 550 nm of 0.25 in aliquots of 1 ml in 1.5-ml microcentrifuge tubes (tube lids were left open). After incubation for 4 h under microaerophilic conditions, 850 µl of bacterial suspension was added to 10 ml of BHIO tempered to 50°C, and 750 µl of this mixture was dispensed into each well of a prepared 12-well plate. Plates were incubated microaerophilically for 30 min while the agar solidified. Subsequently, 10 µl of phage suspension (~10⁹ PFU/ml assayed on propagating strain) was applied to the center of each well. Controls on each plate were a well with only 10 µl of SM buffer added and an untreated well. Plates were incubated microaerophilically for 18 to 36 h and scored for the presence or absence of lysis. Mutants that appeared to have a gain of resistance to at least one phage were rescreened by using the same protocol at least twice more. The determination of transposon insertion sites was as previously described (23).

Motility assays. Motility assays for the phage-type strains and spontaneous mutants was done by the Craigie tube method with 2,3,5-triphenyltetrazolium chloride (TTC) incorporated into motility agar. Viable bacteria reduce the TTC, resulting in a reddish coloration of the media; motile bacteria result in coloration in the outer part of the tube. Motility assays for transposon and defined mutants were performed essentially as described previously (51). Briefly, a platinum wire was inoculated with a single colony and used to stab *Campylobacter* motility agar. Motility was assessed by measuring the colony diameter after incubation at 42°C for 16 h. For large-scale primary screens, 24-well plates were used with 2 ml of motility agar in each well. Confirmatory assays were performed in 90-mm petri dishes containing 30 ml of motility agar, with three inoculation sites: two for the mutant and one for the wild type.

Construction of defined *Campylobacter* mutants. Insertion and insertion-deletion mutants were constructed as follows in the genes *flhA*, required for flagella biosynthesis (9, 36, 40); *kpsC*, required for capsule formation (30); and *pflA*, *motA*, and *motB*, all required for flagellum function (8, 61). Standard methods were used for molecular cloning (47). PCRs were carried out by using an Applied Biosystems GeneAmp PCR System 9700 thermal cycler; reactions were performed in 50-µl volumes containing 0.01 volume of ProofStart DNA polymerase (QIAGEN, Crawley, United Kingdom) according to the manufacturer's instructions with 1 µM concentrations of the forward and reverse primers (Table 2) and the template DNA. Approximately 50 ng of plasmid DNA or 100 ng of genomic DNA was used as a template in the PCR. Thermal cycling conditions were 94°C for 5 min, 30 cycles of 94°C for 1 min (denaturing), 55°C for 1 min (annealing), and 72°C for 1 min (extension), with a concluding extension of 72°C for 10 min. Products were purified by using the QIAquick PCR purification kit (QIAGEN) according to manufacturer's instructions.

To generate the *flhA* mutant, the downstream region, including genes *rpsO* and *cj0883c*, was amplified from *C. jejuni* NCTC 11168 chromosomal DNA by PCR using the primers *ajg302* and *ajg303*. The resulting fragment was cloned as an EcoRI-KpnI fragment into EcoRI-KpnI-digested pUC19 to create the plasmid pAJG180. To facilitate subsequent selections, a chloramphenicol resistance gene with its promoter was PCR amplified from pRY111 using the primers *ajg210* and *ajg211* and inserted as a KpnI-BamHI fragment into KpnI-BamHI-digested pAJG180, generating pAJG181. The upstream region, including *cj0881c* and the last 30 bases of *flhA*, was PCR amplified from *C. jejuni* NCTC 11168 chromosomal DNA using the primers *ajg304* and *ajg305*. The resulting fragment was cloned as a BamHI-PstI fragment into BamHI-PstI-digested pAJG181 to create the plasmid pAJG182. pAJG182 was transformed into *C. jejuni* by natural transformation method using a plate biphasic method adapted from reference 56. The structure of a representative isolate, with a chromosomally located $\Delta flhA::cat$ insertion was confirmed by PCR and Southern hybridization (data not shown).

To create the *kpsC* mutant, the *kpsC* gene was amplified from *C. jejuni* NCTC 11168 chromosomal DNA by PCR using primers *ajg287* and *ajg288*; the resulting fragment was cloned as a BamHI fragment into BamHI-digested pUC19 to create the plasmid pAJG316. To disrupt the reading frame of *kpsC* and to facilitate subsequent selections, a chloramphenicol resistance gene with its promoter was PCR amplified from pRY111 by using the primers *ajg364* and *ajg365* and inserted as a SmaI fragment into SmaI-digested pAJG316, deleting 276 bp of the *kpsC* gene. Restriction analysis confirmed a construct in which the *cat* gene had inserted in the same orientation as *kpsC*. The resulting plasmid, pAJG317, was transformed into *C. jejuni*. The structure of a representative isolate, AG318, with a chromosomally located *kpsC::cat* fusion was confirmed by PCR and Southern hybridization (data not shown).

To create the *pflA* deletion mutant, PCR was used to generate fragments upstream (using primers *jp001* and *jp002*) and downstream (using primers *jp003* and *jp004*) of *pflA*. The upstream region was cloned as a BamHI-SphI fragment into BamHI-SphI-digested pUC19 to create the plasmid pJP1. The downstream

TABLE 1. Bacteria, phages, and plasmids

Strain, bacteriophage, or plasmid	Relevant characteristics ^a	Source or reference ^b
Strains		
<i>E. coli</i>		
DH5 α	F ⁻ (ϕ 80dlacZ Δ M15) Δ (<i>lacZYA-argF</i>)U169 <i>recA1 endA1 hsdR17</i> (r _K ⁻ m _K ⁺) <i>supE44</i> λ ⁻	24
EC100D <i>pir-116</i>	F ⁻ <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) ϕ 80dlacZ Δ M15 Δ <i>lacX74 recA1 endA1 araD139</i> Δ (<i>ara, leu</i>)7697 <i>galU galK</i> λ ⁻ <i>rpsL nupG pir-116</i> (DHFR)	Epicenter Technologies
<i>Campylobacter</i> sp.		
T1	Phage type strain 1, CRU reference no. C605	CRU
T2	Phage type strain 2, CRU reference no. C682	CRU
T5	Phage type strain 5, CRU reference no. C856	CRU
T14	Phage type strain 14, CRU reference no. C10054	CRU
T33	Phage type strain 33, CRU reference no. C1873	CRU
T34	Phage type strain 34, CRU reference no. C13503	CRU
T36	Phage type strain 36, CRU reference no. C1758	CRU
T44	Phage type strain 44, CRU reference no. C10131	CRU
NCTC 11168	Clinical isolate used for genome sequencing	41
NCTC 12658	Propagating strain for Φ 9	CRU
NCTC 12659	Propagating strain for Φ 16	CRU
NCTC 12660	Propagating strain for Φ 7 and Φ 13	CRU
NCTC 12661	Propagating strain for Φ 1 and Φ 2	CRU
NCTC 12662	Propagating strain for Φ 14	CRU
NCTC 12663	Propagating strain for Φ 4 and Φ 12	CRU
NCTC 12664	Propagating strain for Φ 5 and Φ 11	CRU
NCTC 12665	Propagating strain for Φ 6	CRU
NCTC 12666	Propagating strain for Φ 8	CRU
NCTC 12667	Propagating strain for Φ 3	CRU
NCTC 12668	Propagating strain for Φ 10 and Φ 15	CRU
11168 <i>motA::cat</i>	<i>C. jejuni</i> NCTC 11168 with <i>cat</i> cassette in the <i>motA</i> gene; Cm ^r	This study
11168 <i>motB::cat</i>	<i>C. jejuni</i> NCTC 11168 with <i>cat</i> cassette in the <i>motB</i> gene; Cm ^r	This study
11168 Δ <i>pflA::cat</i>	<i>C. jejuni</i> NCTC 11168 in which <i>pflA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
11168 Δ <i>flhA::cat</i>	<i>C. jejuni</i> NCTC 11168 in which <i>flhA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
11168 <i>cj1428c</i>	<i>C. jejuni</i> hypermotile 11168 with <i>cj1428c::kan</i> insertion; Km ^r	-
11168 <i>cj1429c</i>	<i>C. jejuni</i> hypermotile 11168 with <i>cj1429c::kan</i> insertion; Km ^r	-
11168 <i>cj1439</i>	<i>C. jejuni</i> hypermotile 11168 with <i>cj1439::kan</i> insertion; Km ^r	-
11168 <i>kpsM</i>	<i>C. jejuni</i> hypermotile 11168 with <i>kpsM::kan</i> insertion; Km ^r	30
AG318	<i>C. jejuni</i> NCTC 11168 with <i>cat</i> cassette in the <i>kpsC</i> gene; Cm ^r	This study
81-176	Clinical isolate	32
81-176 <i>motA::cat</i>	<i>C. jejuni</i> 81-176 with <i>cat</i> cassette in the <i>motA</i> gene; Cm ^r	This study
81-176 <i>motB::cat</i>	<i>C. jejuni</i> 81-176 with <i>cat</i> cassette in the <i>motB</i> gene; Cm ^r	This study
81-176 Δ <i>pflA::cat</i>	<i>C. jejuni</i> 81-176 in which <i>pflA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
81-176 Δ <i>flhA::cat</i>	<i>C. jejuni</i> 81-176 in which <i>flhA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
M1	Environmental isolate	D. Newell
M1 <i>motA::cat</i>	<i>C. jejuni</i> M1 with <i>cat</i> cassette in the <i>motA</i> gene; Cm ^r	This study
M1 <i>motB::cat</i>	<i>C. jejuni</i> M1 with <i>cat</i> cassette in the <i>motB</i> gene; Cm ^r	This study
M1 Δ <i>pflA::cat</i>	<i>C. jejuni</i> M1 in which <i>pflA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
M1 Δ <i>flhA::cat</i>	<i>C. jejuni</i> M1 in which <i>flhA</i> gene is deleted and replaced by a <i>cat</i> cassette; Cm ^r	This study
Bacteriophages		
Φ 1	UK typing phage 1, NCTC 12673	17
Φ 2	UK typing phage 2, NCTC 12674	17
Φ 3	UK typing phage 3, NCTC 12682	17
Φ 4	UK typing phage 4, NCTC 12676	17
Φ 5	UK typing phage 5, NCTC 12678	17
Φ 6	UK typing phage 6, NCTC 12680	17
Φ 7	UK typing phage 7, NCTC 12671	17
Φ 8	UK typing phage 8, NCTC 12681	17
Φ 9	UK typing phage 9, NCTC 12669	17
Φ 10	UK typing phage 10, NCTC 12683	17
Φ 11	UK typing phage 11, NCTC 12679	17
Φ 12	UK typing phage 12, NCTC 12677	17
Φ 13	UK typing phage 13, NCTC 12672	17
Φ 14	UK typing phage 14, NCTC 12675	17
Φ 15	UK typing phage 15, NCTC 12684	17
Φ 16	UK typing phage 16, NCTC 12670	17

Continued on following page

TABLE 1—Continued

Strain, bacteriophage, or plasmid	Relevant characteristics ^a	Source or reference ^b
Plasmids		
pRY111	Source of <i>cat</i> cassette	60
pAJG180	pUC19 carrying PCR fragment of region upstream of NCTC 11168 <i>flhA</i> ; Ap ^r	This study
pAJG181	pAJG180 carrying <i>cat</i> from pRY111; Ap ^r Cm ^r	This study
pAJG182	<i>flhA</i> deletion construct; pAJG181 carrying NCTC 11168 cj0881c PCR fragment 3' to <i>cat</i> ; Ap ^r Cm ^r	This study
pAJG316	pUC19 carrying NCTC 11168 <i>kpsC</i> gene; Ap ^r	This study
pAJG317	<i>kpsC</i> deletion-insertion construct; pAJG316 with <i>cat</i> cassette inserted into <i>kpsC</i> ; Ap ^r Cm ^r	This study
pJP1	pUC19 carrying PCR fragment of NCTC 11168 region upstream of <i>pflA</i> ; Ap ^r	This study
pJP2	pJP1 carrying PCR fragment of NCTC 11168 region downstream of <i>pflA</i> ; Ap ^r	This study
pBEG000	pUC19 carrying PCR fragment of NCTC 1168 <i>motA</i> gene; Ap ^r	This study
pBEG001	<i>pflA</i> deletion construct; pJP2 with <i>cat</i> cassette inserted into the BamHI site; Ap ^r Cm ^r	This study
pBEG003	<i>motA</i> insertion construct; pBEG000 with <i>cat</i> cassette inserted into the NcoI site of <i>motA</i> ; Ap ^r Cm ^r	This study
pBEG004	pUC19 carrying PCR fragment of NCTC 11168 <i>motB</i> gene; Ap ^r	This study
PBEG005	<i>motB</i> insertion construct; pBEG004 with <i>cat</i> cassette inserted into the XbaI site of <i>motB</i> ; Ap ^r Cm ^r	This study
pUC19	Cloning vector; Ap ^r	59

^a Cm^r, chloramphenicol resistance; Ap^r, ampicillin resistance; Km^r, kanamycin resistance.

^b –, kindly provided by B. Wren, A. Karlyshev, and G. Thacker, London School of Hygiene and Tropical Medicine. CRU, Campylobacter Reference Unit (Health Protection Agency, Colindale, London, United Kingdom).

region was cloned as a BamHI-EcoRI fragment into BamHI-EcoRI-digested pJP1 to create the plasmid pJP2. To facilitate subsequent genetic selections, a chloramphenicol resistance gene with its promoter was PCR amplified from pRY111 using primers jp005 and jp006 and inserted into the BamHI site of pJP2. Restriction analysis confirmed a construct in which the *cat* gene had been inserted in the same orientation as *pflA* would have been. The resulting plasmid, pBEG001, was transformed into *C. jejuni*. The structure of a representative isolate, 11168 Δ*pflA*::*cat*, with a chromosomally located Δ*pflA*::*cat* insertion was confirmed by PCR and Southern hybridization (data not shown).

To create the *motA* mutant, PCR was used to generate a fragment that contained the *motA* gene and flanking sequence. The region was PCR amplified from *C. jejuni* NCTC 11168 chromosomal DNA by PCR using the primers ajg369 and ajg370. The resulting fragment was cloned as an EcoRI-BamHI fragment into EcoRI-BamHI-digested pUC19 to create the plasmid pBEG000. To disrupt the reading frame of *motA* and to facilitate subsequent selections, a chloramphenicol resistance gene with its promoter was PCR amplified from pRY111

using primers ajg371 and ajg372 and inserted into the unique NcoI restriction site within the reading frame of *motA*. Restriction analysis confirmed a construct in which the *cat* gene had been inserted in the same orientation as *motA*. The resulting plasmid, pBEG003, was transformed into *C. jejuni* as previously detailed. The structure of a representative isolate, 11168 *motA*::*cat*, with a chromosomally located *motA*::*cat* fusion was confirmed by PCR and Southern hybridization (data not shown).

To generate the *motB* mutant, the *motB* gene and flanking sequence was PCR amplified from *C. jejuni* NCTC 11168 chromosomal DNA by PCR using the primers ajg373 and ajg395. The resulting fragment was cloned as an EcoRI-SalI fragment into EcoRI-SalI-digested pUC19 to create the plasmid pBEG004. To disrupt the reading frame of *motB* and to facilitate subsequent selections, a chloramphenicol resistance gene with its promoter was PCR amplified from pRY111 using the primers ajg375 and ajg376 and inserted into the XbaI restriction site within the reading frame of *motB* (there are two XbaI sites; however, one was protected from digestion by overlapping *dcm* methylation). Restriction analysis confirmed a construct in which the *cat* gene had been inserted in the same orientation as *motB*. The resulting plasmid, pBEG005, was transformed into *C. jejuni*, and the structure of a representative isolate with a chromosomally located *motB*::*cat* fusion was confirmed by PCR and Southern hybridization (data not shown).

TABLE 2. Oligonucleotides used in this study

Primer	Sequence (5' to 3')
ajg210.....	GGGGGGGGGTACCTCGGCGGTGTTTCCTTTCCAA
ajg211.....	AAAAAAAAGGATCCCGCCCTTTAGTTCCTAAAGGG
ajg287.....	GGGGGGATCCGTGAAGTATTACTCAATATCA
ajg288.....	GGGGGGATCCTCATTTCTTTGCTAAAAATTC
ajg302.....	GAGCTCGAATTCTTATTTATCTCTAAGATTTAA
ajg303.....	CCCGGGGTACCTTATTTTTGCCATTATTTTTTCAT
ajg304.....	GAGCTCGATCCACCGAGGGTATTATTAGGATA
ajg305.....	CCCGGGTGCAGTTATTGTTTTGATTCATTGTT
ajg364.....	GGATCCCCGGGCTCGGCGGTGTTCCCTTTCCAAG
ajg365.....	GAATCCCCGGGTTATTATTCAGCAAGTCTTG
ajg369.....	CCCGGGGAATTCGCGGTTCGCAAGCGTTATT
ajg370.....	CCCGGGGGATCCTTGATATACAGCTGAAACG
ajg371.....	CCCGGGCATGGCTCGGCGGTGTTCCCTTTCCAAG
ajg372.....	CCCGGGCATGGCGCCCTTTAGTTCCTAAAGGG
ajg373.....	CCCGGGGAATTCGCGGTAGAAATCAAGAACC
ajg375.....	CCCGGGTCTAGACTCGGCGGTGTTCCCTTTCCAAG
ajg376.....	CCCGGGTCTAGACGCCCTTTAGTTCCTAAAGGG
ajg395.....	CCCGGGTTCGACGTAGACTTCAAATGGAAGC
jp001.....	GGGGGGCATGCTTTGAAGAATACAAAAGCTTAGAC
jp002.....	CGCGGATCCGTAATTTAGAGATAAAGCTTTTTTG
jp003.....	CGCGGATCCAAAATAATATAGCAAAACTCTTATAAC
jp004.....	CCGGAATCTTTGTGCAATCAAGCTTTCTT
jp005.....	CGCGGATCCCTCGGCGGTGTTCCCTTTCCA
jp006.....	CGCGGATCCCGCCCTTTAGTTCCTAAAGGG

RESULTS

Campylobacter typing phages can be assigned to four groups based on their reaction with spontaneous resistant mutants of Campylobacter type strains. We selected eight of the definitive *Campylobacter* phage-type strains for the isolation of spontaneous mutants resistant to lysis by the typing phage. Seven of these (T1, T2, T5, T33, T34, T36, and T44) were among the most common types identified in the human *Campylobacter* isolates; T14 was also included since this strain is sensitive to all 16 of the typing phages (17). Resistant mutants were selected by spreading a single colony of the type strain onto an agar plate previously impregnated with an individual phage species to which the type strain is sensitive. Five putatively resistant colonies were picked from each plate and subcultured to ensure single isolates were obtained. The resultant strains

TABLE 3. Phage sensitivity of *C. jejuni* phage-type strains and derivatives

Strain and derivative(s) ^a	Phage sensitivity ^b																Motility ^c	HS serotype ^d	PFGE type ^e
	Φ1	Φ2	Φ3	Φ4	Φ5	Φ6	Φ7	Φ8	Φ9	Φ10	Φ11	Φ12	Φ13	Φ14	Φ15	Φ16			
T1				+								+					+	HS2	1
T1Φ4				-								-					-	HS2	1
T1Φ12				-								-					-	HS2	1
T2			+	+				+		+		+		+	+		+	HS4	1
T2Φ3			-	-				-		-		-		-	-		-	HS4	1
T2Φ8			-	-				-		-		-		-	-		-	HS4	1
T2Φ10			-	-				-		-		-		-	-		-	HS4	1
T2Φ4			-	-				-		-		-		-	-		-	HS4	1
T2Φ12			-	-				-		-		-		-	-		-	HS4	1
T5				+	+			+		+		+					+	HS50	2
T5Φ5				+	-			-		-		+					+	HS50	2
T14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	HSUT	3
T14Φ1	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	HS44	3
T14Φ2	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	HS44	3
T14Φ6	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	HS44	3
T14Φ11	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	HS1	3
T14Φ16	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	HS44	3
T14Φ3	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ8	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ10	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ14	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ15	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ5	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	-	HS44	3
T14Φ7	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ9	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	HSUT	3
T14Φ13	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	-	HS8	3
T33	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	HSUT	4
T33Φ1	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HS13	4
T33Φ2	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HS4	4
T33Φ6	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HSUT	4
T33Φ11	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HSUT	4
T33Φ16	+	+		+	+	+	+	+	+	+	+	+	+	-	-	+	+	HS4	4
T33Φ5	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HSUT	4
T33Φ13	-	-		+	+	-	+	+	+	-	+	+	+	-	-	+	+	HS4	4
T34				+	+			+		+		+		+			+	HS50	5
T34Φ16				+	+			+		+		+		+		-	+	HS50	5
T36	+	+		+	+	+	+	+		+		+		+			+	HSUT	6
T36Φ1	-	-		+	+	-	+	+		+		+		+			+	HSUT	6
T44				+						+		+					+	HS31	7
T44Φ10				-						-		-				-	-	HS17	7
T44Φ15				-						-		-				-	-	HS13	7

^a Derivatives are indented in column 1.

^b Scored as lysis observed (+) or not observed (-) in a spot assay using routine test dilution of typing phage.

^c As assessed by using a Craigie tube.

^d Heat-stable serotype as determined by the method of Frost et al. (18). HSUT, untypeable by heat-stable serotype.

^e PFGE was carried out according to the method of Gibson et al. (20); types were defined on the basis of one or more band pattern differences using the method of Peters et al. (43).

were then tested for their reactivity with the typing phage (Table 3). Mutants were designated by the type strain and phage they had been selected on, e.g., T1Φ4 was type strain 1 selected on phage 4. In all cases but T5Φ5, all five mutants isolated from each selection plate showed the same phage resistance phenotype. For T5Φ5 only one of the five isolated mutants showed altered phage sensitivity. Strikingly, common patterns of resistance were observed, in that selection for resistance to one phage also conferred resistance to other phage; e.g., T1Φ4 was resistant to both Φ4 and Φ12, whereas T33Φ1 was resistant to Φ1, Φ2, Φ6, Φ11, and Φ16. These patterns enabled us to group the 16 typing phages into four classes: A (Φ1, Φ2, Φ6, Φ11, and Φ16), B (Φ3, Φ8, Φ10, Φ14, and Φ15), C (Φ5, Φ7, Φ9, and Φ13), and D (Φ4 and Φ12). The only exception was for T33Φ16, which was resistant to Φ16 but not

to any other phage from group A. All of the spontaneous phage-resistant mutants were typed by pulsed-field gel electrophoresis (PFGE) (20) and showed no changes compared to the parent strain (Table 3), indicating that large-scale genomic rearrangements were not responsible for the resistance phenotype. There were six cases, all for which the selecting phage was from group C (T14Φ5, T14Φ7, T14Φ9, T14Φ13, T33Φ5, and T33Φ13), in which the mutant isolated from the selection plate proved upon retesting not to be resistant to the phage used to isolate it but was resistant to phages from other groups (A, B, or D). All variations in sensitivity were stable after storage at -80°C and after repeat subculturing.

Screens of random *C. jejuni* transposon mutants identified classes resistant to lysis by typing phages from different groups. Concurrent with the isolation of spontaneous phage-

TABLE 4. Phage sensitivity of *C. jejuni* NCTC 11168 transposon mutants as assessed by spot assay

Mutant	Resistant to indicated Φ^a	Insertion site	Putative function ^b	Motility
03-21 ^c	1, 2, 6, 16	cj0618	Unknown	+
		cj1413c (<i>kpsS</i>)	Polysaccharide modification protein*	
04-01	1, 2, 6, 16	cj1428c (<i>fcl</i>)	Fucose synthetase*	+
04-05	1, 2, (4), 6, (12), 16	cj1432c	Sugar transferase*	+
04-12	1, 2, (4), 6, (12), 16	cj1432c	Sugar transferase*	+
04-16	1, 2, (4), 6, (12), 16	cj1441c (<i>kfiD</i>)	UDP-glucose 6-dehydrogenase*	+
04-20	1, 2, (4), 6, (12), 16	cj1440c	Sugar transferase*	+
04-23	1, 2, (4), 6, 16	cj0182	Transmembrane transport protein	+
05-03	1, 2, 6, 16	cj1429c	Unknown*	+
31-42	1, 2, 16	cj1532	Unknown	+
45-42	1, 2, 6, 16	cj1438c	Sugar transferase*	+
30-05	(4), 12	cj0171	Unknown	+
30-17	1, 2, 4, 9, 12	cj0390	Unknown	-
30-28	4, 12	cj0860	Integral membrane protein	-
30-39	4, 12	cj0336c (<i>motB</i>)	Flagellar motor protein	-
31-26	4, 12	cj0061c (<i>fliA</i>)	Flagellar biosynthesis sigma factor	-

^a Phages for which variable lysis was seen are indicated in parentheses.

^b From J. Parkhill et al. (41). *, gene in CPS locus.

^c Mutant 03-21 had a transposon insertion in two genes.

resistant mutants, we used a transposon-based mutagenesis approach to determine the genetic basis of *Campylobacter* phage resistance. We screened 400 random transposon mutants of *C. jejuni* NCTC 11168 generated as previously described (23) for resistance to the nine typing phages lytic for this strain (Φ 1, Φ 2, Φ 4, Φ 5, Φ 6, Φ 9, Φ 12, Φ 13, and Φ 16) by a spot lysis method. Fifteen mutants were identified that were refractory to lysis by at least one of the phages. The location of the transposon insertion in these mutants was determined (Table 4). Two major classes of mutants could be identified; strains consistently resistant to Φ 4 and Φ 12 (group D) had insertions in genes known to code for motility functions or were otherwise nonmotile, while eight capsular polysaccharide (CPS) synthesis mutants were resistant to Φ 1, Φ 2, Φ 6, and Φ 16 (group A). Some variable lysis was observed with Φ 4 and Φ 12, a finding consistent with previously published observations (17). In the screen of 400 mutants no resistance was seen to Φ 5 or Φ 13. Because motility functions appeared to be important for sensitivity to Φ 4 and Φ 12, we screened 6,000 transposon mutants for reduced motility and subsequently for sensitivity to the 16 typing phages. A total of 64 motility mutants were identified, and all of them were consistently resistant to Φ 4 and Φ 12 (data not shown). Motility assays were subsequently performed on the spontaneous phage-resistant mutants to determine whether the correlation between the loss of motility in NCTC 11168 transposon mutants and resistance to Φ 4 and Φ 12 also applied to the phage-type strain mutants. All of the wild-type strains were motile, whereas mutants that had gained resistance to Φ 4 and Φ 12 were nonmotile (Table 3). In contrast, mutants that had gained resistance only to phages from group A (Φ 1, Φ 2, Φ 6, Φ 11, and Φ 16) or C (Φ 5, Φ 7, Φ 9, and Φ 13) were motile.

Phage resistance phenotypes of defined motility mutants. To rule out the possibility that the observed phage resistance phenotypes of the NCTC 11168 transposon mutants were due to polar effects of the transposon insertions, defined *pflA*, *flhA*, *motA*, and *motB* mutants were constructed in the NCTC 11168 background. All of these strains were nonmotile (not shown)

and were resistant to group D phages (Φ 4 and Φ 12) as determined by spot assay, confirming the results from the transposon screens. In the screens of the *Campylobacter* phage-type strains, resistance to group B phages (Φ 3, Φ 8, Φ 10, Φ 14, and Φ 15) was always accompanied by resistance to Φ 4 and Φ 12 and loss of motility. This suggested that there may be a connection between sensitivity to group B phages and motility. NCTC 11168 of phage type 38 is not sensitive to Φ 3, Φ 7, Φ 8, Φ 10, Φ 11, Φ 14, or Φ 15; hence, no information regarding factors required for lysis by group B phages could be obtained from the transposon screens of this strain. By the spot assay, we determined that *C. jejuni* 81-176 (32) was sensitive to Φ 3, Φ 5, Φ 10, and Φ 14 and that strain M1 was sensitive to Φ 3 and Φ 14 (not shown). We constructed defined *pflA*, *flhA*, *motA*, and *motB* mutants of these two strains, confirmed that they were nonmotile, and tested their phage sensitivity by spot assay. All of these motility mutants were resistant to lysis by the group B phages (data not shown).

Heat-stable antigen serotyping of *Campylobacter* phage-type strain mutants. CPS is the determinant of heat-stable antigen serotype (11, 18, 30). The correlation between gain of resistance to phages from group A and transposon-mediated mutation of CPS genes suggested that an HS serotype change may be observed in the spontaneous mutants resistant to these phages. Serotyping (Table 3) showed that to some extent this was the case; of 14 mutants that had gained resistance to phages from group A, 9 (64%) had an altered serotype. In contrast, 4 of 19 (21%) mutants that had gained resistance only to phages from group B, C, or D showed a serotype change.

Phage resistance phenotype of defined CPS mutants. Because the NCTC 11168 transposon library screening implicated CPS biosynthetic genes as being important in sensitivity to group A phages (Φ 1, Φ 2, Φ 6, and Φ 16), we sought to confirm the phenotypes of some of the transposon mutants by screening defined mutants of NCTC 11168 in *cj1428c* (*fcl*) and *cj1429c*. In both cases the defined mutants had a wild-type phenotype as determined by the spot assay. Possibly, the transposon insertions were polar on downstream genes, resulting in

a significant alteration in, or complete loss of, the capsule structure in these mutants compared to the defined mutants. If this was the case, we predicted that acapsular mutants would be resistant to $\Phi 1$, $\Phi 2$, $\Phi 6$, and $\Phi 16$. To investigate this, we screened defined mutants in *kpsC* (constructed in the present study), *kpsM*, and *cj1439* (*glf*), previously shown to be acapsular (30, 52) for phage sensitivity using the spot assay. All three mutants were completely resistant to the lytic activity of $\Phi 1$ and $\Phi 2$. For $\Phi 6$ and $\Phi 16$, the mutants were sensitive but to a lesser degree than the wild type.

DISCUSSION

Despite the significance of *C. jejuni* as a food-borne pathogen, comparatively little is known regarding the physiology, biochemistry, or pathogenesis of the organism. *Campylobacter* sp. incidence in the United Kingdom is high; over 50,000 laboratory cases were confirmed each year from 1997 to 2001 in England and Wales, although there has been a decrease to around 42,000 cases annually over recent years (26). The use of phage typing in conjunction with serotyping facilitates the screening of large numbers of isolates (17). The key to the continued understanding of the significance of *Campylobacter* spp. in food safety is the ability to distinguish between strains. This is necessary for the identification of sources of infection and elucidation of the routes of transmission and is a prerequisite for the development of targeted control strategies.

With the continued emergence of antibiotic-resistant bacteria and the desire to identify alternative methods to control pathogens, the specificity of phage for particular bacterial species has led some researchers to propose the exploitation of phage as a possible means to control *Campylobacter* spp. (2, 3, 13, 21, 35, 57). For this to be a viable option, it will be necessary to have a greater understanding of the relationship between *C. jejuni* and *Campylobacter*-specific phages. In the present study, we sought to investigate these phage-bacterium interactions by screening for spontaneous and transposon mutants resistant to lysis by the United Kingdom typing phages. These phages were selected since they are the best-characterized *Campylobacter*-specific phages; data regarding their ultrastructure, genome size, and restriction profile is available (45), as are their patterns of lysis against many *Campylobacter* isolates (17). Sixteen phages are used in the United Kingdom typing scheme, all of which belong to the *Myoviridae* family, and they have been classified into three groups based on structural criteria: group I ($\Phi 4$ and $\Phi 12$), group II ($\Phi 3$, $\Phi 8$, $\Phi 10$, $\Phi 14$, and $\Phi 15$), and group III ($\Phi 1$, $\Phi 2$, $\Phi 5$, $\Phi 6$, $\Phi 7$, $\Phi 9$, $\Phi 11$, $\Phi 13$, and $\Phi 16$) (45).

We screened eight *Campylobacter* phage-type strains for spontaneous gain of resistance to the typing phages and observed common patterns of resistance that enabled us to group the phages into four categories: group A ($\Phi 1$, $\Phi 2$, $\Phi 6$, $\Phi 11$, and $\Phi 16$), group B ($\Phi 3$, $\Phi 8$, $\Phi 10$, $\Phi 14$, and $\Phi 15$), group C ($\Phi 5$, $\Phi 7$, $\Phi 9$, and $\Phi 13$), and group D ($\Phi 4$ and $\Phi 12$). Selection for resistance to phages from within a group in general also conferred resistance to the other phages within that group, presumably as a consequence of phages within a group targeting related bacterial factors. Screens of transposon and defined mutants of *C. jejuni* NCTC 11168, M1, and 81-176 showed that resistance to phages from group A could be imparted by disruption of CPS expression, while loss of motility correlated

with resistance to phages from groups B and D. Moreover, this was reflected in the screens of spontaneous mutants, where resistance to phage groups B and D was also associated with a motility defect. Interestingly, there was a correlation between the structural groupings reported by Sails et al. (45) and the phenotypic groupings reported here; phages with a motility association, those in groups B and D, correspond to structural groups II and I, respectively, whereas the CPS-associated phages are all in structural group III. Whether these groupings are a general feature of *Campylobacter* phages is unclear. It would be of interest to determine whether additional phage species, such as those recently isolated from retail poultry (2, 13, 15), show these structural and phenotypic relationships.

In the screens for spontaneous mutants there were a few exceptions to these rules. Phage-type strain T33 selected on $\Phi 16$ yielded a derivative resistant to $\Phi 16$ but sensitive to other phages from group A. Given the apparent connection between CPS and phages from this group, it is possible that this spontaneous resistant mutant had a CPS structural change that specifically disrupted the interaction with $\Phi 16$ but did not affect the ability of the other phages to bind to the bacterium. Nuclear magnetic resonance and mass spectroscopy techniques such as have recently been used to characterize the CPS structure of *Campylobacter* (52, 53) could be used to investigate this possibility, but such was beyond the scope of the present study. In six cases of the screens for spontaneous phage-resistant mutants, all using phages from group C, the isolated derivative proved upon retesting to be sensitive to the selecting phages but resistant to phages from other groups. Possibly, the initial selection was not stringent enough to select for mutants resistant to direct application of high-titer phages. The resistance of these isolates to phages from groups A, B, and D may suggest that resistance to these phages arises at a relatively high frequency or imparts some limited protection against group C phages.

Successful phage replication requires binding of the phage to the host bacterium, injection and replication of phage DNA, synthesis of progeny phage particles, and lysis of the host. The initial binding necessarily requires an interaction between the phage and structures on the surface of the bacterium. It has long been known that flagella and extracellular polysaccharide can serve as phage receptors in other bacterial species (33). For flagellatropic phages, e.g., *Bacillus subtilis* phage PBS1 (44), *E. coli* χ -phage (28, 49), and Φ CbK of *Caulobacter crescentus* (5), the flagella are not thought to be the site at which irreversible attachment of the phages to the bacteria occurs. Rather, the phage appears to reversibly bind to the flagella and use its rotation to translocate to the bacterial body, where irreversible attachment and DNA injection occur (33, 44, 48, 49). Bacteria with intact but paralyzed flagella are refractory to lysis by such phages (33, 48). Our results are consistent with this model; resistance to phages from groups B and D was observed for defined mutants in *flhA*, which are aflagellate (9, 36, 40), as well as *motA*, *motB*, and *pflA* mutants (8, 61), which possess intact but nonfunctional flagella. The association between motility and phage in *Campylobacter* is significant in light of the fact that the *Campylobacter* flagellum is known to be phase variable (34, 40). Variation in flagellum expression would be predicted to result in inconsistent patterns of lysis with flagellatropic phages, and indeed Frost et al. reported that

in the development of the United Kingdom phage typing scheme the reactions exhibited by $\Phi 4$ and $\Phi 12$ were difficult to reproduce (17). We also observed this phenomenon during screens of transposon mutants.

The structure of the *Campylobacter* CPS has recently been determined for strain NCTC 11168 (52). CPS is a determinant of sensitivity in some phage-bacterium systems (33). In the present study NCTC 11168 *kpsC*, *kpsM*, and *cj1439* mutants, previously shown to be acapsular (30, 52), were resistant to lysis by United Kingdom typing phages $\Phi 1$ and $\Phi 2$. These phages show very similar reactions in the typing scheme (17) and have similar restriction digest patterns (45). Lysis by the other phages from group A lytic for NCTC 11168 ($\Phi 6$ and $\Phi 16$) appeared to be reduced but not eliminated for these acapsular mutants. Further characterization of the molecular basis of the phage-CPS interaction will be required to account for these differences. Expression of the capsule of *Campylobacter* is thought to be phase variable (29, 41), although there does not appear to be the same variation in the degree of lysis with $\Phi 1$, $\Phi 2$, $\Phi 6$, and $\Phi 16$ as with the flagellatrophic $\Phi 4$ and $\Phi 12$. Variable regions in the CPS locus are associated with structural genes rather than the transport functions that display CPS on the cell surface (30, 34, 41). Presumably, phase variation in this region results in alterations of CPS structure rather than switching between capsulated and acapsular forms. Indeed, variation of CPS structure within a strain of *Campylobacter* has been observed (1, 25, 53). This contrasts with the flagella, the expression of which is known to be phase variable (34, 40). It may therefore be expected that resistance to $\Phi 4$ and $\Phi 12$ would arise at a higher frequency than that for group A phages.

We have demonstrated that the United Kingdom *Campylobacter* typing phages fall into four classes based on their reactivity with mutants of the type strains and NCTC 11168. Group A phages ($\Phi 1$, $\Phi 2$, $\Phi 6$, $\Phi 11$, and $\Phi 16$) may interact with the capsule, whereas phages of groups D ($\Phi 4$ and $\Phi 12$) and B ($\Phi 3$, $\Phi 8$, $\Phi 10$, $\Phi 14$, and $\Phi 15$) may be flagellatrophic. Interestingly, the results for one transposon *cj0390* mutant suggested that there may be some overlap between the phage groups. This nonmotile mutant was resistant to $\Phi 4$ and $\Phi 12$ (group D) but also to $\Phi 1$ and $\Phi 2$ (group A) and $\Phi 9$ (group C). *cj0390*, which is predicted to possess a transmembrane domain and a number of tetratricopeptide repeats that are believed to mediate protein-protein interactions, was previously identified in motility screens of *C. jejuni* transposon mutants (12; A. Grant, unpublished observations). The precise function of *cj0390* in bacterial motility is the subject of a continued study (A. Kanji and A. Grant, unpublished observations) and will be published elsewhere. The lack of motility would account for the resistance to $\Phi 4$ and $\Phi 12$. The resistance to $\Phi 1$, $\Phi 2$, and $\Phi 9$ could be due to pleiotropic effects of the mutation or may indicate overlap of the receptors for phages from different groups. This study did not give any other indication regarding bacterial factors important in sensitivity to phages from group C ($\Phi 5$, $\Phi 7$, $\Phi 9$, and $\Phi 13$).

The phase-variable nature of flagella and capsule means that at least 12 of the 16 typing phages may be interacting with targets that can vary within a strain of *Campylobacter*, calling into question the efficacy of the phage-typing scheme as currently described. Particularly notable in this regard is the

dominance of PT1, accounting for 19.6% of *C. jejuni* isolates from human infections (17), defined on the basis of sensitivity to only phages $\Phi 4$ and $\Phi 12$. The flagellatropic nature of these phages may account for the inconsistent lysis patterns previously seen with them (17) and makes a case for their removal from the typing scheme. Frost et al. suggested this as a possibility but noted that the removal of these phages would increase the proportion of untypeable isolates from 15 to 35% (17). Extension of the phage typing scheme by the addition of new phages could improve discrimination, provided any new phages did not target phase-variable structures. More likely, however, is the increased use of genetic techniques such as multilocus sequence typing (14) and comparative phylogenomics (10), which also yield data regarding the evolutionary relationships between different strains, in contrast to the phenotypic methods of phage typing and serotyping.

The use of phage as a control strategy for *Campylobacter* spp. is still of great interest, but a more detailed understanding of the phage-bacterium interaction is of paramount importance before it can be used successfully. Recent studies by Connerton and coworkers have begun to explore the dynamics of phage-host interaction during colonization of chickens with *Campylobacter* spp. (13, 15, 35). The present study has begun to characterize the molecular basis of the phage-host interaction and demonstrates that consideration must be made of the possibility that phage targets are under phase-variable regulation. The significance of this observation is likely to be different depending upon where the use of phage therapy is envisaged. Application of phage in vivo, within broiler chickens prior to slaughter, for example, is fundamentally different from post-processing applications, where the bacteria may not be replicating. A recent report has shown that in an experimental model, phage-resistant campylobacters were isolated at a lower frequency in vivo (from chicken intestinal contents) than in vitro (broth culture) (35). These resistant bacteria became sensitive to phage when passaged through the avian host. Since structural features such as flagella and CPS are important in colonization (4, 23, 27, 37, 38, 42, 54, 58), it is tempting to speculate that phase variation gives rise to a population that is phage resistant but impaired for colonization. Whatever the precise dynamics of the phage-host interaction, it is likely that any phage-based intervention strategy would use combinations of phage that simultaneously target the capsule, flagella, and, ideally, nonvariable structures, too, should such phage be identified. We are currently extending our screens of transposon mutants to explore new host-phage interactions, as well as consolidating our findings from the present study to identify the actual receptors required by the phages for their binding.

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