Abstract

Campylobacter jejuni is an important cause of bacterial diarrhea worldwide. The pathogenesis of C. jejuni is poorly understood and complicated by phase variation of multiple surface structures including lipoooligosaccharide, capsule, and flagellum. When C. jejuni strain 81-176 was plated on blood agar for single colonies, the presence of translucent, non-motile colonial variants was noted among the majority of opaque, motile colonies. High-throughput genomic sequencing of two flagellated translucent and two opaque variants as well as the parent strain revealed multiple genetic changes compared to the published genome. However, the only mutated open reading frame common between the two translucent variants and absent from the opaque variants and the parent was motA, encoding a flagellar motor protein. A total of 18 spontaneous motA mutations were found that mapped to four distinct sites in the gene, with only one class of mutation present in a phase variable region. This study exemplifies the mutative/adaptive properties of C. jejuni and demonstrates additional variability in C. jejuni beyond phase variation.

Introduction

Campylobacter is one of the most common sources of diarrheal disease in developing as well as developed countries [1]. In the United States, Campylobacter is the second leading cause of foodborne illness among pathogens monitored by the Foodborne Diseases Active Surveillance Network, or FoodNet [2]. Infection with Campylobacter, or campylobacteriosis, is a self-limiting disease associated with a variety of symptoms that range from watery diarrhea to dysentery accompanied with fever. In addition, Campylobacter jejuni infection has been linked to the development of post-infectious sequelae including Guillain-Barré syndrome [3,4], reactive arthritis [3], and irritable bowel syndrome [5–7]. Associations with inflammatory bowel disease [8,9] and Celiac disease [10] have also been suggested.

The first published C. jejuni genome sequence, that of strain NCTC11168, revealed reversible phase variation in genes encoding surface antigens mediated by slip strand mismatch (SSM) repair at homopolymeric tracts of 8 or more Gs or Cs [11]. Many of these GC tracts are located in genes involved in the biosynthesis of surface structures including the polysaccharide capsule and lipoooligosaccharide (LOS), both of which have been demonstrated to be phase variable [12]. Phase variation has also been reported to affect shorter poly-A tracts located in flgR and flgS, a two-component system that regulates expression of C. jejuni flagella [13,14]. Thus, it is thought that phase variation is a mechanism whereby the bacteria can modify the antigenic makeup of its surface to evade the host immune system or adapt to new hosts or environments. The frequency of phase variation in C. jejuni ranges from approximately $1 \times 10^{-3}$ to $4 \times 10^{-5}$ mutations/division and is dependent on the length of the homopolymeric tract [15]. SSM errors are attributed to the absence of a functional methyl-directed DNA mismatch repair system (MMR) in C. jejuni [11].

Strain 81-176, first isolated during a 1981 outbreak of acute enteritis in Minnesota associated with consumption of contaminated raw milk [16], is one of the best characterized strains of C. jejuni. Strain 81-176 has been demonstrated to invade human epithelial cell lines at high levels in vitro [17,18], to be virulent in human [19,20] and primate models of diarrheal disease [21,22], to be amenable to genetic analysis, and has been sequenced by two independent groups [23,24]. Here we describe colonial variation of C. jejuni strain 81-176 that is associated with loss of motility. Sequence comparison of five derivatives of strain 81-176 from the same lineage revealed several changes from the published genome [24]. Among the five derivatives sequenced were two non-motile, translucent variants, both of which were mutated in motA, a gene necessary for energy transmission to the flagellum and subsequent motility. Further characterization of the translucent phenotype indicated a general association with loss of motility. Random sequencing of the motA gene of 56 additional translucent variants indicated that 29% of these were motA mutations and that the mutations occurred at 4 distinct sites in the gene. These included SSM at a homopolymeric tract, a deletion, an insertion, and a transversion. Collectively, the data underscore the genomic instability of C. jejuni.
Results

Appearance of colonial variants

The lineages of 81-176 are shown in Figure 1A. Strain 81-176/55 was isolated as single, encapsulated colony from the cGMP-stock of 81-176 that was sequenced by The Institute for Genomic Research (TIGR) [24]. When strain 81-176/55 was plated for single colonies on Campylobacter Blood Agar (CBA), two distinct colonial variants were observed (Figure 1B). These variants were opaque (O), appearing as a slightly larger light/white colonies, or translucent (T) colonies having a darker hue. The frequency with which the T variants appeared amongst the population of strain 81-176/55 was on average 17% (range 0–50%). Given that colonial variation in C. jejuni has been linked with motility [26], variant colonies were tested in soft agar (Figure 1C). While the O colonies were motile, the three T colonies tested were non-motile and lacked darting motility in wet mounts. Motility is also known to be phase variable [13,14] and thus expression of FlgR, FlgS, and flagelin was examined by immunoblot in the T variants (data not shown; [25]). All three colonies expressed FlgR, FlgS, and flagelin, and appeared flagellated by transmission electron microscopy (Figure 1E). Several genes known to be associated with a paralyzed flagella filament (pflA, pflP, pflQ) were PCR-amplified and sequenced, but no sequence changes were evident (data not shown).

Whole genome sequencing

In an effort to identify the genotypic cause of the non-motile, flagellated variants, two O (O1 and O3) and two T (T1 and T3) variant progeny, as well as the 81-176/55 parent, were sequenced using Illumina technology (Figure 1A). Alignment of the 81-176/55 parent sequence to the published sequence [24] also allowed comparison of two lineages of 81-176 with relatively few passages between them. When the sequences were assembled and compared to the published reference 81-176 sequence [24], varying numbers of mutations were revealed throughout the genome. Beyond the expected variation in homopolymeric tracts, single nucleotide polymorphisms (SNPs) and indels (short insertions or deletions) located mostly in intergenic regions were identified (Table S1). One of these variations was identified between C[J]81176_0920 (encoding CysK, cysteine A biosynthesis protein) and C[J]81176_1731 (encoding hup, a histone-like DNA-binding protein). Conventional sequencing confirmed a deletion of 69 bp in the intergenic region in front of C[J]81176_0920 (cysK; Figure 2). This deletion, while present in 81-176/55 and all four variant offspring, was not observed in the 81-176 reference sequence or the whole genome sequencing performed by Hofreuter et al. [23]. The deletion may have occurred between an imperfect direct repeat of 7 bp (Figure 2).

The only mutated open reading frame common to both sequenced T variants but absent from the O variants, 81-176/55, and the two published 81-176 sequences was C[J]81176_0359, encoding the flagellar motor protein MotA. Interestingly, the two sequenced T variants each had distinct mutations in motA. Variant T1 had a G to C transversion at base pair 262 (labeled B in Figure 3) that resulted in an A87P mutation (Figure 4). Variant T3 had a SNP at base 612 that resulted in a G5 to G4 tract change (labeled D in Figure 3) and led to a truncation of 53 amino acid residues in the C-terminus of MotA (Figure 4).

Association of the T phenotype with loss of motility

To further confirm the role of motA mutation in the appearance of the T variants, a motA insertional inactivation mutant was generated and shown to have a ‘translucent’ phenotype comparable to what was seen for the spontaneous T variants (data not shown). However, insertional mutants in flagellin and pflA also displayed the T phenotype (data not shown). Thus, the T phenotype appeared associated with loss of motility by a variety of mutations, and the two that were picked for genomic sequencing happened to both contain a mutated motA gene.

Colonial variation on other media and by other lineages of 81-176

To determine if O/T variation was specific to CBA plates, 81-176/55 was plated for single colonies on Mueller-Hinton (MH) agar. Instead of O and T variation, distinct and spreading colonies were observed (Figure 1D), similar to what has been previously described for Campylobacter [26–28]. Furthermore, when plated to MH, the T variants isolated on CBA appeared as distinct colonies and the O variants appeared as spreading colonies. Thus, for simplicity, all colonial variants having either a translucent or distinct phenotype will be referred to as T whereas the spreading and opaque variants will be referred to as O. Colonial variation was not unique to 81-176/55 as a variety of 81-176 lineages, including cGMP 81-176 (the parent strain of clone #55) [20] and 6 laboratory stocks frozen at different times, were also observed to form T and O colonies. Overall, the T variants occurred amid a population at a frequency of ~20% regardless of lineage.

Incidence of flgR and/or flgS mutants among T variants

Since flgR and flgS, encoding a two component system regulating flagella expression, are phase variable [13,14], we examined twenty non-motile T variants to determine expression of FlgR and/or FlgS by immunoblotting [25]. The results indicated that three colonies (15%) were FlgR– FlgS+, three colonies (15%) were FlgR+ FlgS+, 10 colonies (50%) were FlgR FlgS+, and only 4 colonies (20%) were FlgR+ FlgS–.

Sequencing of motA in additional T variants

Non-motile T variants from different lineages of strain 81-176 were isolated from blood agar, MH, or Blaser-Wang (BW). The motA gene was PCR amplified and sequenced from a total of 56 individual non-motile colonies, each from independent platings to avoid characterization of sibling colonies. Most of the colonies sequenced expressed a wildtype motA gene (40/56 or 71%) and were presumably mutated in flgR and/or flgS. A total of 16 colonies (29%) were motA mutants and the mutations observed fell into four classes, two of which had been observed in the two original T variants. The four classes were called Type A, B, C, or D (Figure 3 and Table 1). Eight additional variants exhibited the G5 to G4 change exemplified by the T3 mutant (Type D mutation). Four additional variants had an A87P change similar to the previously sequenced T1 mutant (Type B mutation). The third mutation observed occurred in three variants and was found to be a duplication of 49 bp that created a direct repeat within motA (Type C mutation, represented in the T108 mutant). The consequence of the duplication event was to replace the last 73 residues in the C-terminus with a sequence of 39 new residues (Figure 4). Finally, a single variant, T158, with a deletion of five nucleotides at base pairs 64-68 (Type A mutation), was found that created a nonsense mutation likely resulting in the use of an alternative start codon and the generation of a truncated protein (Figure 4). Representative mutants of Type B (T1), Type C (T108), and Type D (T3) were incubated in semi-soft motility agar (Table 1) to determine if the mutations were revertable; the single Type A mutant was unavailable for testing. Motile forms of each mutant were isolated from the motility agar and sequence analysis of the motA gene of each demonstrated restoration of the wildtype sequence.
Discussion

Phase variation is thought to provide *C. jejuni* an adaptive advantage, aiding in virulence and/or survival. The high degree of genomic variability exhibited by *C. jejuni* isolates results from horizontal gene transfer, intragenomic rearrangements, and the presence of homopolymeric repeats within the genome [29]. Stretches of nucleotide repeats prone to mispairing during DNA replication have been described to abound within the genome of *C. jejuni* [11,13] and the frequency of variation due to SSM is known to vary based on the length of the repetitive unit [15]. *C. jejuni* lacks the prototypical MMR of *E. coli* [11,24] which functions...
to correct single base mismatches or short nucleotide runs that escape proofreading during DNA replication [30]. A defect in MMR has been demonstrated to contribute to SSM [31,32] and a hypermutator phenotype in a number of bacteria [31,33–35]. Thus, it is likely that the absence of MMR facilitates the genetic diversity seen in C. jejuni.

Indeed, C. jejuni has been suggested to be unstable in culture. Strain NCTC11168 has varying phenotypes; different lineages display transcriptional differences and varying virulence capabilities in vitro and in vivo [36–39]. Re-sequencing of NCTC11168 lineages and comparison to NCTC11168-GS, the original sequence generated in 2000 [11], revealed extensive variation in contingency loci with generally fewer SNPs/indels outside these regions [38–40]. Re-sequencing following mouse-adaptation [38] or human infection [41] also revealed changes primarily within contingency genes.

Figure 2. Schematic illustration of the 69 bp region missing in 81-176/55 and its 4 additional sequenced offspring. The assembly of the whole genome sequences revealed varying SNPs throughout the chromosome. One confirmed difference between the previously sequenced 81-176 and 81-176/55 (as well as the opaque and translucent progeny) was the presence of a 69 bp deletion in the intergenic region between hup and cysK. An incomplete direct repeat (IDR) bracketing the deletion is indicated.

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Strain 81-176 has been previously sequenced by two independent groups, both of which received the strain from the NMRC (Figure 1A). The first whole genome sequencing, by Hofreuter et al. at Yale University, was performed by high-throughput sequencing using 454 sequencing technology [23]. The second whole genome sequencing was performed by conventional dideoxy sequencing at TIGR [24]. Both strains were derived from the same stock of 81-176, but were of different lineages (Figure 1A). Although no comparison of the two sequences has been published, a direct alignment revealed many single nucleotide deletions. Many of these differences are likely due to the different technologies used for sequencing. Here we had the opportunity to compare five related isolates of 81-176–two T variants, two O variants, and the parent of both–using the same sequencing technology. Despite minimal passage of the strains, sequence variation was evident as shown in Table S1. 81-176/55 and all derivatives had a 69 bp deletion in the intergenic region in front of hup and cysK.

Figure 3. Nucleotide alignment of motA and mutated alleles. The four mutations found within motA have been assigned a type for ease in distinguishing. Type A mutation is a deletion at base 64 (T158). Type B mutation is a missense mutation at base 262 (T1). Type C mutation is a duplication of 49 bp (boxed) creating a direct repeat (T108). Type D mutation is a nonsense mutation at base 612 (T3).

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Spontaneous motA Mutations in C. jejuni 81-176
CJJ81176_0920 compared to the TIGR sequence. This deletion was not present in the 81-176 strain sequenced at Yale, which was derived from the NMRC laboratory stock. The 69 bp deletion most likely affects the transcriptional start site (TSS) of cysK. CysK, the enzyme encoded by CJJ81176_0920, is involved in the biosynthesis of L-cysteine from L-serine. It remains unclear as to how this deletion happened, but an imperfect direct repeat (TTTTTAA and TTATTAA) of seven base pairs flanks the deleted region (Figure 2).

In the current study, the presence of translucent, non-motile variants of 81-176/55 was detected in a population of opaque, motile bacteria. Although the two original T variants sequenced were motA mutants, the non-motile population was composed of both flagellated and non-flagellated forms. Random sequencing of the motA gene of 56 translucent colonies revealed that 16 (29%) were mutated in this gene. Thus, a total of 18 independent spontaneous motA mutations were sequenced and the mutants fell into 4 distinct classes, only one of which could be considered classical SSM at a homopolymeric tract. Interestingly, motile revertants to 3 of these variant classes were readily detected by plating onto semi-soft motility agar; the only representative with a type A mutation was not tested.

In this study we demonstrate a link between colonial variation and motility in strain 81-176. It remains to be determined if similar variations occur in other C. jejuni strains. Although phase variation of motility [13,42] and colonial variation of C. jejuni [26,27] have both been described, an association between colony morphology and motility in C. jejuni has not, to the best of our knowledge, been reported previously. In the related species C. coli, Park et al. reported on the appearance of large, swarming colonies and smaller, pin-point colonies [28]. The different colony morphologies of C. coli were linked to variation from a flagellated to non-flagellated form due to phase variation of a homopolymeric T tract in flhA, a tract not found in the flhA gene of C. jejuni [13]. Hendrixson previously characterized multiple spontaneous changes in flgS using a genetic screen [14] and the breadth of changes described here for motA are reminiscent of those described for flgS. In flgS, the deletion of a duplicated ACCTT run resulted

Table 1. Sequenced T (translucent or distinct) variants with a motA mutation listed by type (see Figure 3).

| Type | Mutation | Position | Sequence | Representative | # of Variants (%) | Rev² |
|------|----------|----------|----------|----------------|------------------|-----|
| A    | Deletion | 64       | TTAGA    | T158           | 1 (5)            | N/D |
| B    | SNP      | 262      | G→C (A→P) | T1             | 5 (28)           | Yes |
| C    | Insertion| 559      | 49 bp    | T108           | 3 (17)           | Yes |
| D    | Deletion | 612      | G tract  | T3             | 9 (50)           | Yes |

¹Percentage of variants out of 18 total.
²Reversion of the mutation confirmed by sequence analysis. N/D indicates that analysis was not done for this single variant.

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in premature termination of the protein. Here, a deletion of a TTAGA near the 5' end of motA (Type A mutation) resulted in a nonsense mutation the consequence of which is likely the use of an alternative start codon truncating the protein. Additionally, the duplication of a 49 bp sequence in motA (Type C mutation) resulted in premature termination of the protein. Indeed, mutation of motA occurred by a variety of mechanisms only one of which, the SNP at base 612 that results in the deletion of a single guanine nucleotide within a homopolymeric tract (Type D), is similar to classical phase variation described to occur via an infrequent homopolymeric G tracts located within the AT-rich genome [11,13,14].

MotA and MotB form the stator complex of the flagella that provides energy to the flagellar motor by conducting the flow of protons [43]. The 258-residue MotA protein shares 24% (56/234) identity and 44% (104/234) similarity with the E.coli counterpart (Figure S1A). Prediction of transmembrane helices [44] allowed identification of a similar conformation for C. jejuni MotA in comparison to the E.coli protein (Figure S1B). Both proteins contain two inner membrane domains with two large cytoplasmic domains. Based on comparison with the MotA of E. coli, predictions can be made regarding the effect of the 4 mutations within motA on C. jejuni protein function. Both Type C and Type D nonsense mutations result in premature protein termination; the deletion of the C-terminus region of MotA in these mutants is likely to render the protein non-functional as this region is localized in the cytoplasm and is crucial for torque generation [43]. An additional nonsense mutant, Type A mutation, occurs early in translation likely resulting in the use of alternative start codons and production of a truncated protein sequence missing the N-terminus 39 amino acids. The remaining mutation, Type B, is a missense mutation whereby a SNP confers an A87P change. Mutational studies in E. coli have demonstrated that deletion or deletion of proline residues in MotA was detrimental to torque generation [43]. In particular, four residues of MotA were critical for its functionality: Arg90, Glu98 (cytoplasmic domain), Pro173, and Pro222 (interface between the cytoplasmic domain and the membrane domain). Studies in different bacterial species point out the role of charged residues (Glu and Arg) in the function of the stator as well as the role of proline in the conservation of the conformation [45]. As proline residues have a crucial role in keeping the conformation of MotA optimal [43], the introduction of an additional proline in Type B mutants most likely disturbs the final conformation of the protein rendering it non-functional.

The ability to phase vary flagellar expression by SSM of fliR/S reduces the energy required to synthesize the filament, but an advantage to high frequency mutation of a motor gene in a bacterium expressing a full-length filament is not obvious. C. jejuni is known to secrete a variety of non-flagellar proteins through the flagellar filament in the absence of a specialized type three secretion system, some of which are thought to play a role in virulence or commensalism [46–49]. A motA4 mutant retained the capacity to secrete at least one of these proteins, FspA (data not shown) [47]. Maintaining functionality of the flagellar export apparatus/flagellar secretion system may provide a benefit to motA mutants over other flagellar mutants such as fliR or fliS that are unable to secrete proteins due to the lack of expression of the flagellar structure [50].

The current study of strain 81-176 demonstrated genetic variations beyond the expected phase variations, including the deletion of an intergenic region after limited passage in vitro as well as genetic variation comparable to that seen for differing lineages of NCTC11168 [38,41]. Such genetic diversity amongst the C. jejuni population likely facilitates adaptation to the different environments encountered by this zoonotic human pathogen. While the re-sequencing data obtained herein, and that reported for NCTC11168, both demonstrate the genetic variation of C. jejuni, the current study links variation to a non-motile form with the appearance of colonial variants. Overall, the data shed light on the extent of genetic variation and instability seen for C. jejuni that complicates the investigation of this pathogen.

Materials and Methods

Bacterial strains and growth conditions

C. jejuni strains 81-176 (HS23/36) has been described previously [11,23]. The flac flaB::Cm mutant of 81-176 has also been previously described [25,51]. The NMRC laboratory strain of 81-176 was originally derived from a volunteer fed the original strain at the Centers for Vaccine Development [19]. A stock of this strain was prepared at the Walter Reed Army Institute of Research Bioproduction Facility in 1994 under current Good Manufacturing Practices (cGMP) for use in clinical trials [20]. More recently the cGMP stock was plated for single colonies that were screened by immunoblot to identify colonies producing polysaccharide capsule [52] for other studies. One such encapsulated colony, 81-176/55, was expanded and used in this study (Figure 1A). For identification of variants, strains were plated at an appropriate dilution to observe ~100 colonies/plate on Campylobacter Blood Agar (CBA; BBLCampy-BAP, BD, Sparks, MD). Otherwise, strains were routinely grown on Mueller-Hinton (MH) agar plates incubated in microaerobic atmosphere (85% N2, 10% CO2, 5% O2) at either 37°C or 42°C for 18 to 24 hours. Remel CVA plates (Thermo Fischer, Lenexa, KS), porcine brain heart infusion, MH supplemented with Blaser-Wang (BW; Oxoid Limited, Hampshire, England), and Brucella broth (BB) agar were additionally used for bacterial growth.

Motility assays

Wet mounts were performed by examining viable bacteria, resuspended in PBS, by light microscopy. Single colonies picked from CBA plates were stabbed into semisoft motility agar, either MH 0.4% agar petri plates or BB 0.6% agar 24-well plates [53]. Growth occurred at 37°C under microaerobic conditions and plates were analyzed for motility by a plus/minus system after ≥24 hours.

Transmission electron microscopy

For visualization of flagella, bacteria were grown in T25 biphasic cultures. The supernatant was removed, washed, and used as the sample for staining and imaging. The bacterial samples were negatively stained with uranyl acetate and images were taken on a JEOL-100 CX II transmission electron microscope (JOEL Ltd., Peabody, MA) at the NMRC Research Services Directorate, Pathology Department (Silver Spring, MD).

Immunoblot analyses

Whole cell preparations of C. jejuni were subjected to SDS-PAGE analysis on 12.5% tris-glycine gels, transferred to nitrocellulose, and blotted for flagellin and the phase variable regulators FlgR and FlgS using rabbit polyclonal antisera with a goat anti-rabbit secondary conjugated to HRP as previously described [25]. Blots were developed colorimetrically using BCIP/NBT (Pierce, Rockford, IL).

Directed analysis of genetic content

Individual flagellar genes were PCR amplified from bacterial boil preps of non-motile variants using a proof-reading polymerase
Whole genome sequencing

DNA from 81-176/55 and four variants was isolated using phenol chloroform extraction and subjected to high-throughput genomic sequencing on the Hi-Seq platform (Illumina). Genomic sequencing libraries were constructed according to standard operating procedures at The Institute for Genome Sciences Genome Resource Center (http://www.igs.umaryland.edu/resources/grc/index.php). These methods have been utilized to generate genome data for over 1000 bacterial isolates (http://gcid.igs.umaryland.edu/).

Sequence read mapping and annotation

Raw sequence reads were mapped to the reference genome, C. jejuni 81-176 (accession number CP000538.1), using Mira v3.4.0 [34]. Sequence and structural variants were identified and manually verified from the Mira output. The raw sequence data were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (Study accession # SRS463970).

Validation of sequence differences by conventional sequencing

Following high-throughput genomic sequencing, regions of uncertainty were re-sequenced by conventional DNA sequencing methods. Forward and reverse sequencing primers (Table S2) were designed using the online Primer3 software [35]. Sequencing reactions were performed and assembled as described above. Similarly, the motA allele of variants was amplified by PCR (see Table S2 for primers used) and the amplicon was sequenced as described previously.

Generation of motA insertional mutants

The motA gene was amplified from 81-176 such that BamHI restriction sites would flank the amplicon (see Table S2 for primers used). The PCR product was gel purified, ligated into BamHI-digested pBluescript, and transformed into DH5α. The kanamycin (Km) resistance gene aph3 from plasmid pILL600 [56,57] was cloned as a Smal fragment into a unique NcoI site within motA located 466 bp from the ATG start of the 777 bp gene. The plasmid containing motA::aph3 was electroporated with selection on Km into C. jejuni strain 81-176. Clones were screened with primers that bracketed the insertion point of the Km cassette to confirm a double crossover.

Generation of pflA mutant

A previously described pUC18 plasmid containing a large region of pflA with a kanamycin resistance cassette (aph3; previously described) inserted between the BcII sites within pflA (pRY301) [58] was purified from DH5α cells. The purified plasmid was electroporated into strain 81-176 and the resultant Km β clone was screened for insertion by PCR using primers for pflA amplification (Table S2). 81-176/pflA::aph3 was verified as non-motile in motility agar.

Phenotypic assessment of mutants

To ascertain the phenotype of 81-176 mutants, bacteria were harvested from MH plates and mixed with an 81-176 O variant. The mixture was diluted, plated on blood agar for colonial variants, and incubated for 3 days at 37°C. Single T and O colonies were picked and analyzed for antibiotic resistance by plating on MH with/without antibiotics and for motility in 0.6% semi-soft agar.

Supporting Information

Figure S1 Effects of mutation on MotA. (A) Comparison of putative conserved residues between MotA of E. coli and C. jejuni. Residues critical for functionality include Arg90, Glu98, Pro173, and Pro222; these are highlighted for E. coli with similar residues marked on the C. jejuni MotA as putatively having comparable roles. The alanine undergoing change to proline in Type B mutation is indicated in bold for C. jejuni. (B) Comparison of predicted transmembrane domains of E. coli and C. jejuni MotA. Prediction of transmembrane helices was performed using TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Table S1 Summary of mutations identified in the five newly sequenced C. jejuni 81-176 clones compared to the C. jejuni 81-176 reference sequence. The first column lists the gene/region affected by a mutation. The second column lists the function of the gene or intergenic region of the mutation. The third column specifies if the mutation occurred in a known GC tract. Column 4 indicates the consequence of the mutation on the predicted amino acid sequence. The sequence of mutations in intergenic regions is unknown. Column 5 indicates the type of mutation (SNP, single nucleotide polymorphism). Column 6 lists the nucleotide changes and base pair affected on the reference genome.

Table S2 This table lists the oligonucleotide primers used in this study.

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Author Contributions

Conceived and designed the experiments: KLM FP PG. Performed the experiments: KLM FP JWS. Analyzed the data: KLM FP JWS DAR PG. Contributed reagents/materials/analysis tools: KLM FP JWS DAR. Wrote the paper: KLM FP JWS DAR PG.
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