Published in final edited form as:
Curr Opin Biotechnol. 2009 April ; 20(2): 158–165. doi:10.1016/j.copbio.2009.03.004.

Comparative studies of *Campylobacter jejuni* genomic diversity reveal the importance of core and dispensable genes in the biology of this enigmatic food-borne pathogen

Tri Duong and **Michael E. Konkel***
School of Molecular Biosciences, Washington State University, 406B Abelson Hall, PO Box 644234, Pullman, WA 99164

Summary of recent events

MLST, DNA microarrays, and genome sequencing has allowed for a greater understanding of the metabolic capacity and epidemiology of *Campylobacter jejuni*. While strain-specific genes may provide an isolate a selective advantage in environments and contribute to the organism's pathogenicity, recent work indicates that *C. jejuni* pathogenicity is dictated by variations in the nucleotide sequence of core genes. Challenges facing *C. jejuni* researchers include determining: a) the degree to which genomic diversity enables this bacterium to persist in particular environments; b) if *C. jejuni* virulence and disease severity can be predicted based on genotype; c) the set of core and variable genes whose products contribute to virulence; and d) the genes in which nucleotide changes can affect a strain's pathogenicity.

Introduction

Campylobacter jejuni, a Gram-negative, spiral shaped bacterium [1], is one of the leading bacterial causes of food-borne human gastroenteritis. *C. jejuni* is currently estimated to cause 5 to 14% of diarrhea worldwide, which translates into 400−500 million cases per year [2]. Most cases of *C. jejuni* mediated gastroenteritis (campylobacteriosis) are characterized by nausea, abdominal cramps, diarrhea and fatigue. Although campylobacteriosis is most often self-limiting, certain strains of *C. jejuni* have been implicated as an antecedent to the development of Guillain-Barré Syndrome (GBS), an acute autoimmune mediated polyneuropathy characterized by ascending paralysis [3,4].

While outbreaks of campylobacteriosis occur, predominantly through consumption of contaminated milk and untreated water [5], most *Campylobacter* infections are sporadic in nature and linked to the improper handling and consumption of poultry. The linkage between human infection and the handling of raw poultry is not unexpected, as *C. jejuni* is a common commensal organism of chickens. In fact, *C. jejuni* colonize the intestinal tract of a variety of animals, including common livestock (cattle, sheep, pigs), domestic animals (dogs, cats), poultry, and wildlife (rabbits, pheasant, quail) [6-9]. A number of methods [e.g., Penner serotyping, Lior serotyping, *fla*-short variable region (SVR) sequencing, pulsed-field gel

*Corresponding Author: Michael E. Konkel, School of Molecular Biosciences, Washington State University, 406 B Abelson Hall, PO Box 644234, Pullman, WA 99164 Phone: (509)335−5039 Fax: (509)335−1907 Email: konkel@wsu.edu.

electrophoresis (PFGE), multilocus sequence typing (MLST)] are useful for the discrimination of *C. jejuni* isolates in epidemiological investigations. These methods have enabled investigators to identify the strain responsible for an outbreak [10,11]. The use of MLST, in particular, has provided researchers with the benefit of a defined molecular fingerprint to compare strains. The recent explosion of genome sequences and comparative genomic data has increased our understanding of the epidemiology and metabolic capacity of this organism.

The number of *Campylobacter* species and strain-specific genome sequences is increasing

The importance of *Campylobacter* in human gastrointestinal illness has resulted in at least eighteen isolates from eight different *Campylobacter* species having been or being sequenced (Table 1). The genomes of *Campylobacter* organisms are characterized by a low mol% (G+C) content (between 29.5% and 44.5%), small size (ranging from 1.5 Mb for *Campylobacter lari* RM2100 to 2.1 Mb for *Campylobacter concisus* 13826), and relatively few open reading frames (between 1425 and 1931 ORFs). The availability of sequenced genomes has aided in the development of methodologies to address questions concerning the relationship between genomic content and *C. jejuni* biology.

MLST provides greater insight into genetic diversity and population structure

MLST allows researchers to differentiate strains based on alleles at seven “unlinked” housekeeping loci [12]. Each unique allele is assigned a number based on its order of discovery and the combination of allelic numbers is the basis for its sequence type (ST). The ST is indicative of the isolate's genotype. This method is advantageous because it yields data that are accurate, reproducible, unaffected by changes in gene order, and readily comparable among laboratories. In addition to its usefulness in investigating *C. jejuni* outbreaks, MLST has demonstrated that *C. jejuni* are genetically diverse. Dingle *et al.* [12] found 194 *C. jejuni* isolates to be genetically diverse with a weakly clonal population structure. The genetic diversity was evident in that of the 155 STs observed, 104 STs could be grouped into 11 major genetic lineages or clonal complexes whereas the remaining 51 STs were unique. Manning *et al.* [13] performed MLST on 266 *C. jejuni* veterinary and human isolates and found that the populations overlapped among the 19 clonal complexes identified. Dingle *et al*. [14] applied the MLST scheme used for *C. jejuni* to *Campylobacter coli* and found the two species to share approximately 86% nucleotide sequence identity at the housekeeping loci and found some evidence for horizontal gene transfer. The investigators found additional evidence for horizontal gene transfer by sequencing the short variable region (SVR) of the *flaA* flagellin gene [14]. Evidence for horizontal gene transfer was also reported by Meinersmann *et al*. [15] using *flaA* SVR sequence anlaysis. These studies demonstrate that recombination between *C. jejuni* and *C. coli* occurs, and supports a hypothesis that these two species are continuing to evolve [16]. Taken together, these studies demonstrate the genetic diversity of *Campylobacter* strains. While it may never be possible to predict an isolate's pathogenicity or an individual's clinical symptoms or disease severity based on the ST alone, the use of MLST has already contributed to a greater understanding of *C. jejuni* population structures and their relationships with a variety of hosts.

Identification of hypervariable plasticity regions

The availability of genome sequences has made it possible to construct whole genome DNA microarrays for comparative genomic hybridization (CGH) analysis. Analysis of 11 *C. jejuni* clinical isolates by CGH revealed extensive genetic diversity and enabled the researchers to identify the genetic core of this organism [17]. Approximately 84% of the 1654 genes analyzed were common to all strains tested and encoded proteins involved in housekeeping functions,

including metabolic, biosynthetic, cellular and regulatory processes. Strain-specific gene differences were involved in the biosynthesis and modification of cell surface structures including flagella, lipooligosaccharide (LOS) and capsular polysaccharide. Pearson *et al.* [18] examined the genomic diversity of 18 *C. jejuni* strains from different sources and found that the variable genes were often present in clusters, suggesting they were acquired or lost in groups during evolution [18]. In particular, seven hypervariable plasticity regions (PR) (Figure 1) were identified (PR1 – PR7). PR1 contained genes important in the utilization of alternative electron acceptors during respiration, possibly conferring a selective advantage in restricted oxygen environments. PR2, PR3 and PR7 contained genes encoding outer membrane and periplasmic proteins, which may be linked to phenotypic variation and adaptation to different ecological niches. PR4, PR5 and PR6 contained genes involved the biosynthesis and modification of flagella, LOS, and capsule. Further studies are required to elucidate the contribution of genes within the hypervariable regions to a strain's phenotype.

Meta-analysis of CGH data from four separate data sets revealed that many of the variable genes were absent or divergent in only one of the 97 strains [19]. In a separate study, phylogenomic analysis of 111 isolates from human, animals, and environmental sources provided insight into the population structure of *C. jejuni*. The investigators identified livestock and non-livestock associated clades (Figure 2A) [20]. Although analysis of *C. jejuni* isolates recovered from individuals with different clinical symptoms did not cluster (Figure 2B), more than half of the clinical isolates were phylogenomically related to strains from non-livestock sources. The investigators concluded that environmental sources serve as an important reservoir for *C. jejuni* infectious isolates. In contrast, Wilson *et al.* [21] concluded that livestock is the principal source of *C. jejuni* infection based on modeling DNA sequence evolution and rates of zoonotic transmission [21]. They suggested that the frequency of recombination in *C. jejuni* makes a single phylogenetic tree an inappropriate representation of the relationship between *C. jejuni* genomes [21]. Based on these studies, it is apparent that additional work is needed to determine the relative importance of reservoirs for *C. jejuni*.

C. jejuni genomes are syntenic and some contain integrated elements

Presumably, all *C. jejuni* strains sequenced to date are pathogenic. As such, it is not possible to compare the genomic sequence of pathogenic and non-pathogenic strains. However, sequencing and comparative genomic analysis of five *Campylobacter* genomes (*C. jejuni* NCTC 11168, *C. jejuni* RM1221, *C. coli* RM2228, *C. lari* RM2100, and *Campylobacter upsaliensis* RM3195) revealed major structural differences between the strains [22]. While the genome of *C. jejuni* RM1221 was syntenic with the previously sequenced *C. jejuni* NCTC 11168, it contained four inserted genomic islands termed *Campylobacter jejuni*-integrated elements (CJIEs) (Figure 1). The CJIEs from *C. jejuni* RM1221 were not present among the other three *C. jejuni* sequenced strains (NCTC 11168, 81116, and 81−176). CJIE1 was found to be a *Campylobacter* Mu-like phage while CJIE2 and CJIE4 contained genes predicted to encode phage related endonucleases, methylases and repressors. A total of 73% of the CJIE3 predicted proteins showed sequence similarity with those encoded on the *C. coli* RM2228 megaplasmid, suggesting that it may be an integrated plasmid. Comparative genomic analysis of 67 *C. jejuni* and 12 *C. coli* strains from various geographical, clinical and veterinary sources revealed the CJIEs widely distributed, and more than half of the strains tested contained at least one CJIE [23]. *C. coli* RM2228 was also highly syntenic with *C. jejuni* RM1221, while *C. lari* and *C. upsaliensis* were not [22]. Noteworthy is that the regions of sequence variability aligned with the previously identified PR in *C. jejuni* NCTC 11168, suggesting the PR are likely physical loci within *C. jejuni* genomes. Additionally, CJIEs 2 and 4 appeared at loci adjacent to PR 2 and 5, respectively. The relevance of the CJIEs in the biology of *C. jejuni* remains to be determined.

Comparative genomic analyses have helped identify dispensable genes amongst *C. jejuni* isolates (i.e., genes absent or highly divergent in one or more of the isolates). The genes unique among the four *C. jejuni* sequenced strains (NCTC 11168, RM1221, 81−176 and 81116) are listed in Supplemental Table 1. The majority of these genes mapped to the variable loci identified previously and encode hypothetical proteins (Figure 3). Some of the unique genes were predicted to be involved in capsular polysaccharide biosynthesis, DNA modification, lipoprotein synthesis, or were phage-related. Additionally, the DNA sequences flanking the variable loci were conserved, suggesting that large regions of the *C. jejuni* chromosome are relatively stable. Sequencing of *C. jejuni* 81−176 revealed aerobic and anaerobic respiratory pathways that may confer advantages in low oxygen environments (i.e., gastrointestinal tract) [24]. Additionally, an ortholog of γ-glutamyltranspeptidase was identified, which is important for *Helicobacter pylori* colonization [25,26]. A mutation of this gene, resulting in loss of function, significantly reduced *C. jejuni* colonization of mice [24]. Together, these findings indicate that strain variable genes can provide an isolate an advantage in selective environments.

Nucleotide variations alter pathogenic behavior

The availability of *C. jejuni* genome sequences has provided the genetic basis for investigating the metabolism, gene regulation, and physiology of these organisms. These data indicate that many of the previously identified putative virulence determinants, including cytolethal distending toxin (CDT), various adhesins (e.g., CadF, JlpA, and PEB1), and the flagellar structural proteins, are conserved amongst strains [17]. Despite conservation of these genes, it is likely that the variations in the pathogenicity of *C. jejuni* strains are influenced by nucleotide differences in virulence genes. In this regard, none of the techniques discussed above are capable of determining whether point mutations, nucleotide insertions, nucleotide deletions, and gene rearrangements are present in one strain versus another. Below we provide specific examples of where nucleotide variations may affect the pathogenicity of *C. jejuni* strains.

Comparative analysis of CDT, a multi-subunit toxin [27,28], in *C. jejuni*, *C. coli*, and *Campylobacter fetus* revealed that the *cdt* gene cluster is widely distributed in a species-specific manner [29]. CDT has been shown to induce tissue damage and fluid accumulation in the colon of infected mice [30]. However, the contribution of CDT in campylobacteriosis is not clear, as *C. jejuni* CDT-negative strains have been isolated from individuals with clinical signs of diarrhea [31]. Detection of the *cdtABC* gene cluster does not necessarily result in the expression and synthesis of a functional product [31]. AbuOun *et al*. [31] identified two mutations that result in a CDT-negative phenotype. One mutation was characterized by a 667 bp deletion, encompassing a portion of both the *cdtA* and *cdtB* genes, and the second mutation was a nucleotide change that resulting in nonsynonymous residue at position 95 of CdtB [31]. This example illustrates the importance of performing experiments to test for a functional product even when a gene is detected by hybridization.

While not well understood, single nucleotide variations may contribute to molecular mimicry of gangliosides concentrated in peripheral nervous tissue by the LOS of *C. jejuni* [32,33]. Although CGH analyses of *C. jejuni* strains from individuals with GBS and uncomplicated gastroenteritis have failed to identify GBS associated genes [34], direct sequence analysis has revealed information regarding phase variation in the LOS biosynthesis genes [35]. Analysis of the genome sequence of *C. jejuni* 81−176 identified a homopolymeric G tract in *cgtA*, which encodes *N*-acetylgalactosaminyltransferase. Variation in the number G residues resulted in differences in the LOS core structure, affecting ganglioside mimicry and the ability of this strain to invade epithelial cells *in vitro* [35]. This example illustrates that slipped-strand mispairing occurs during DNA replication at homopolymeric tracts consisting of consecutive guanine residues. The resulting differences in homopolymeric tract length can affect translation

and result in phase variation. Given that these regions are frequently associated with genes that synthesize surface structures [22,24,36] their presence may influence a strain's pathogenicity and immunogenicity. Lastly, it is worth noting that although homopolymeric G tracts are common in the *C. jejuni* strains sequenced, their number varies among strains.

Comparison of the *C. jejuni* NCTC 11168 (11168-GS) isolate used for generating the genomic sequence and the original clinical isolate (11168-O) revealed differences in their virulence-associated phenotypes including colonization, invasion, translocation and motility [37]. Gene expression profiling of these two strains revealed dramatic differences in the expression of flagellar and motility related genes. Sequence analysis of three sigma factors (i.e., RpoD, RpoN, and FliA) in the 11168-GS and 11168-O strains identified single-nucleotide polymorphisms (SNPs) in each sigma factor gene, resulting in at least one amino acid substitution in each sigma factor. The investigators proposed that the differences in gene expression were due to changes in global gene regulation. The possibility that a nucleotide change in a sigma factor could influence gene expression on a global level was subsequently proven by the identification a defective *rpoN* gene in *C. jejuni* strain S2B and its complementation [38].

Malik-Kale *et al.* [38] assessed the virulence potential of two *C. jejuni* strains (Turkey and CS) that were indistinguishable by PFGE, MLST, and CGH. Interestingly, these two *C. jejuni* strains showed dramatically different virulence potential in both *in vitro* cell culture and piglet models. Gene expression analysis revealed dramatic differences in gene expression profile. In particular flagellar Class II genes were found to have lower expression in the *C. jejuni* CS strain (i.e., the less virulent isolate), suggesting expression of these genes are important to pathogenesis by *C. jejuni*. Additionally, DNA sequence analysis of genes that regulate flagellar synthesis revealed a point mutation in *flgR* may be responsible for the loss of virulence. Previous work indicated that flagellar synthesis is modulated via phase variation [39]. In particular, phase variation of FlgR is due to the loss or gain of a nucleotide in homopolymeric adenine or thymine tracts within *flgR*. The identified point mutation in *flgR* did not occur in a region previously identified as being phase variable. Based on these data, we conclude that allelic variation may also dictate a strain's pathogenic potential.

Conclusions and future perspectives

The identification of genetic markers predictive of ecological source and virulence potential are important to detecting and preventing the dissemination of *C. jejuni* via food sources. As we have reviewed, MLST, DNA microarrays, and genome sequencing of *C. jejuni* strains have demonstrated the genetic diversity of this important food-borne pathogen. Comparative genomic studies have demonstrated *C. jejuni* population structure relates to ecological source (livestock versus non-livestock sources) and identified genes (e.g., Cj1321−1326 in *C. jejuni* NCTC 11168) that are associated with livestock sources. Additionally, DNA sequence analyses implicate phase and allelic variation as possible mechanisms for altered gene expression and protein synthesis. In spite of recent advances, significant gaps exist in our knowledge of *C. jejuni* biology. First, researchers have yet to uncover a correlation between genomic diversity and disease severity. Second, *C. jejuni* virulence and disease pathology are not yet predictable based on genotype. Third, the core genes necessary for disease and the variable (i.e., dispensable) genes whose products contribute to *C. jejuni* disease are not known. Fourth, based on the observation that nucleotide changes in certain genes alter a strain's pathogenicity, studies are needed to identify additional genes/proteins whose expression/function is influenced by nucleotide/residue variations. To address these questions, a small infectious disease animal model is needed to test the pathogenic potential of *C. jejuni* isolates. However, few animal models are currently available to assess *C. jejuni* virulence [40]. One possibility is the use of the interleukin-10-deficient murine model to determine the relationship between *C. jejuni*

genetic variation and disease spectrum [41]. Continued work focusing on the relationship of genotype to phenotype is important in understanding this enigmatic organism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Charles L. Larson (School of Molecular Biosciences, Washington State University) and Dr. Philip F. Mixter (School of Molecular Biosciences, Washington State University) for critical review of this manuscript. Comparative genomic analyses of *C. jejuni* is supported from funds awarded to MEK from the National Institute of Health, Department of Health and Human Services under contract number NO1-AI-30055.

References

1. Miller, WG.; Mandrell, RE. Prevalence of *Campylobacter* in the food and water supply: Incidence, Outbreaks, Isolation and Detection.. In: Ketley, JM.; Konkel, ME., editors. Campylobacter jejuni: Molecular and Cellular biology. Horizon Bioscience; 2005. p. 101-164.
2. Friedman, CR.; Neimann, J.; Wegener, HC.; Tauxe, RV. Epidemioogy of *Camylobacter jejuni* infections in the United States and other industrialized nations.. In: Nachamkin, I.; Blaser, MJ., editors. Campylobacter. Vol. 2nd. ASM Press; 2000. p. 121-138.
3. Nachamkin I, Allos BM, Ho T. *Campylobacter* species and Guillain-Barre syndrome. Clin Microbiol Rev 1998;11:555–567. [PubMed: 9665983]
4. Nachamkin, I.; Allos, BM.; Ho, TW. *Campylobacter jejuni* infection and the association with Guillain-Barre syndrome.. In: Nachamkin, I.; Blaser, MJ., editors. Campylobacter. Vol. 2nd. ASM Press; 2000. p. 155-175.
5. Pebody RG, Ryan MJ, Wall PG. Outbreaks of *Campylobacter* infection: rare events for a common pathogen. Commun Dis Rep CDR Rev 1997;7:R33–37. [PubMed: 9080726]
6. Atanassova V, Ring C. Prevalence of *Campylobacter spp*. in poultry and poultry meat in Germany. Int J Food Microbiol 1999;51:187–190. [PubMed: 10574094]
7. Koene MG, Houwers DJ, Dijkstra JR, Duim B, Wagenaar JA. Strain variation within *Campylobacter* species in fecal samples from dogs and cats. Vet Microbiol 2009;133:199–205. [PubMed: 18678447]
8. Soncini G, Valnegri VL, Vercellotti L, Colombo F, Valle D, Franzoni M, Bersanii C. Investigation of *Campylobacter* in reared game birds. J Food Prot 2006;69:3021–3024. [PubMed: 17186674]
9. Stern NJ, Bannov VA, Svetoch EA, Mitsevich EV, Mitsevich IP, Volozhantsev NV, Gusev VV, Perelygin VV. Distribution and characterization of *Campylobacter* spp. from Russian poultry. J Food Prot 2004;67:239–245. [PubMed: 14968953]
10. Gerner-Smidt P, Hise K, Kincaid J, Hunter S, Rolando S, Hyytia-Trees E, Ribot EM, Swaminathan B. PulseNet USA: a five-year update. Foodborne Pathog Dis 2006;3:9–19. [PubMed: 16602975]
11. Klena, JD.; Konkel, ME. Methods for Epidemilogical Analysis of *Campylobacter jeuni*.. In: Ketley, JM.; Konkel, ME., editors. Campylobacter: Molecular and Cellular Biology. Horizon Bioscience; 2005. p. 165-179.
12. Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, Bootsma HJ, Willems RJ, Urwin R, Maiden MC. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol 2001;39:14–23. [PubMed: 11136741]
13. Manning G, Dowson CG, Bagnall MC, Ahmed iH, West M, Newell DG. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl Environ Microbiol 2003;69:6370–6379. [PubMed: 14602588]
14. Dingle KE, Colles FM, Falush D, Maiden MC. Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. J Clin Microbiol 2005;43:340–347. [PubMed: 15634992]

15. Meinersmann RJ, Phillips RW, Hiett KL, Fedorka-Cray P. Differentiation of *Campylobacter* populations as demonstrated by flagellin short variable region sequences. Appl Environ Microbiol 2005;71:6368–6374. [PubMed: 16204559]

16. Sheppard SK, McCarthy ND, Falush D, Maiden MC. Convergence of *Campylobacter* species: implications for bacterial evolution. Science 2008;320:237–239. [PubMed: 18403712]

17. Dorrell N, Mangan JA, Laing KG, Hinds J, Linton D, Al-Ghusein H, Barrell BG, Parkhill J, Stoker NG, Karlyshev AV, et al. Whole genome comparison of *Campylobacter jejuni* human isolates using a low-cost microarray reveals extensive genetic diversity. Genome Res 2001;11:1706–1715. [PubMed: 11591647]

18. Pearson BM, Pin C, Wright J, I'Anson K, Humphrey T, Wells JM. Comparative genome analysis of *Campylobacter jejuni* using whole genome DNA microarrays. FEBS Lett 2003;554:224–230. [PubMed: 14596944]Using Array-CGH of 18 C. jejuni isolates from diverse sources, the authors were able to identify hypervariable plasticity regions (PRs). This is the first microarray based comparsion to include non-human isolates.

19. Taboada EN, Acedillo RR, Carrillo CD, Findlay WA, Medeiros DT, Mykytczuk OL, Roberts MJ, Valencia CA, Farber JM, Nash JH. Large-scale comparative genomics meta-analysis of *Campylobacter jejuni* isolates reveals low level of genome plasticity. J Clin Microbiol 2004;42:4566–4576. [PubMed: 15472310]

20. Champion OL, Gaunt MW, Gundogdu O, Elmi A, Witney AA, Hinds J, Dorrell N, Wren BW. Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. Proc Natl Acad Sci U S A 2005;102:16043–16048. [PubMed: 16230626]Using array-CGH, the authors were able to classify 111 C. jejuni isolates from diverse clinical, animal and environmental sources into either a livestock or non-livestock clade and identify genes associated with sources of isolation. This is the first genomic comparison to identify genes associated with an isolates source.

21. Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle PJ. Tracing the source of campylobacteriosis. PLoS Genet 2008;4:e1000203. [PubMed: 18818764]

22. Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, Brinkac LM, DeBoy RT, Parker CT, Daugherty SC, et al. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. PLoS Biol 2005;3:e15. [PubMed: 15660156]Sequencing and comparison of C. jejuni RM1221, C. lari RM2100, C. upsalienis RM3195 and C. coli RM2228. This is the first whole genome sequence comparison to include non-jejuni Campylobacter species.

23. Parker CT, Quinones B, Miller WG, Horn ST, Mandrell RE. Comparative genomic analysis of *Campylobacter jejuni* strains reveals diversity due to genomic elements similar to those present in *C. jejuni* strain RM1221. J Clin Microbiol 2006;44:4125–4135. [PubMed: 16943349]

24. Hofreuter D, Tsai J, Watson RO, Novik V, Altman B, Benitez M, Clark C, Perbost C, Jarvie T, Du L, et al. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect Immun 2006;74:4694–4707. [PubMed: 16861657]

25. Chevalier C, Thiberge JM, Ferrero RL, Labigne A. Essential role of *Helicobacter pylori* gamma-glutamyltranspeptidase for the colonization of the gastric mucosa of mice. Mol Microbiol 1999;31:1359–1372. [PubMed: 10200957]

26. McGovern KJ, Blanchard TG, Gutierrez JA, Czinn SJ, Krakowka S, Youngman P. gamma-Glutamyltransferase is a *Helicobacter pylori* virulence factor but is not essential for colonization. Infect Immun 2001;69:4168–4173. [PubMed: 11349094]

27. Muza-Moons MM, Koutsouris A, Hecht G. Disruption of cell polarity by enteropathogenic *Escherichia coli* enables basolateral membrane proteins to migrate apically and to potentiate physiological consequences. Infect Immun 2003;71:7069–7078. [PubMed: 14638797]

28. Yamasaki S, Asakura M, Tsukamoto T, Faruque SM, Deb R, Ramamurthy T. Cytolethal distending toxin (CDT): genetic diversity, structure and role in diarrheal disease. Toxin Reviews 2006;25:61.

29. Asakura M, Samosornsuk W, Taguchi M, Kobayashi K, Misawa N, Kusumoto M, Nishimura K, Matsuhisa A, Yamasaki S. Comparative analysis of cytolethal distending toxin (cdt) genes among *Campylobacter jejuni, C. coli* and *C. fetus* strains. Microb Pathog 2007;42:174–183. [PubMed: 17353111]

30. Okuda J, Fukumoto M, Takeda Y, Nishibuchi M. Examination of diarrheagenicity of cytolethal distending toxin: suckling mouse response to the products of the *cdtABC* genes of *Shigella dysenteriae*. Infect Immun 1997;65:428–433. [PubMed: 9009292]

31. Abuoun M, Manning G, Cawthraw SA, Ridley A, Ahmed IH, Wassenaar TM, Newell DG. Cytolethal distending toxin (CDT)-negative *Campylobacter jejuni* strains and anti-CDT neutralizing antibodies are induced during human infection but not during colonization in chickens. Infect Immun 2005;73:3053–3062. [PubMed: 15845513]

32. Ang CW, Laman JD, Willison HJ, Wagner ER, Endtz HP, De Klerk MA, Tio-Gillen AP, Van den Braak N, Jacobs BC, Van Doorn PA. Structure of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity and clinical features of Guillain-Barre and Miller Fisher patients. Infect Immun 2002;70:1202–1208. [PubMed: 11854201]

33. Yuki N. Infectious origins of, and molecular mimicry in, Guillain-Barre and Fisher syndromes. Lancet Infect Dis 2001;1:29–37. [PubMed: 11871407]

34. Leonard EE 2nd, Tompkins LS, Falkow S, Nachamkin I. Comparison of *Campylobacter jejuni* isolates implicated in Guillain-Barre syndrome and strains that cause enteritis by a DNA microarray. Infect Immun 2004;72:1199–1203. [PubMed: 14742576]

35. Guerry P, Szymanski CM, Prendergast MM, Hickey TE, Ewing CP, Pattarini DL, Moran AP. Phase variation of *Campylobacter jejuni* 81−176 lipooligosaccharide affects ganglioside mimicry and invasiveness in vitro. Infect Immun 2002;70:787–793. [PubMed: 11796612]

36. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, et al. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 2000;403:665–668. [PubMed: 10688204]

37. Gaynor EC, Cawthraw S, Manning G, MacKichan JK, Falkow S, Newell DG. The genome-sequenced variant of *Campylobacter jejuni* NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. J Bacteriol 2004;186:503–517. [PubMed: 14702320]

38. Malik-Kale P, Raphael BH, Parker CT, Joens LA, Klena JD, Quinones B, Keech AM, Konkel ME. Characterization of genetically matched isolates of *Campylobacter jejuni* reveals that mutations in genes involved in flagellar biosynthesis alter the organism's virulence potential. Appl Environ Microbiol 2007;73:3123–3136. [PubMed: 17369342]Using two genotypically indistinguisible C. jejuni isolates with dramatically different virulence potentials as determined by in vitro and in vivo models, the authors were able to identify a single nucleotide substitution responisble for differences in virulence potential.

39. Hendrixson DR. A phase-variable mechanism controlling the *Campylobacter jejuni* FlgR response regulator influences commensalism. Mol Microbiol 2006;61:1646–1659. [PubMed: 16899076]

40. Konkel, ME.; Monteville, MR.; Klena, JD.; Joens, LA. *In vitro* and *in vivo* models used to study *Campylobacter jejuni* virulence properties.. In: Torrence, ME.; Isaacson, RE., editors. Microbial Food Safety in Animal Agriculture. Iowa State Press; 2003. p. 195-210.

41. Mansfield LS, Bell JA, Wilson DL, Murphy AJ, Elsheikha HM, Rathinam VA, Fierro BR, Linz JE, Young VB. C57BL/6 and congenic interleukin-10-deficient mice can serve as models of *Campylobacter jejuni* colonization and enteritis. Infect Immun 2007;75:1099–1115. [PubMed: 17130251]

42. Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 2004;14:1394–1403. [PubMed: 15231754]

43. Pearson BM, Gaskin DJ, Segers RP, Wells JM, Nuijten PJ, van Vliet AH. The complete genome sequence of *Campylobacter jejuni* strain 81116 (NCTC11828). J Bacteriol 2007;189:8402–8403. [PubMed: 17873037]

44. Poly F, Read T, Tribble DR, Baqar S, Lorenzo M, Guerry P. Genome sequence of a clinical isolate of *Campylobacter jejuni* from Thailand. Infect Immun 2007;75:3425–3433. [PubMed: 17438034]

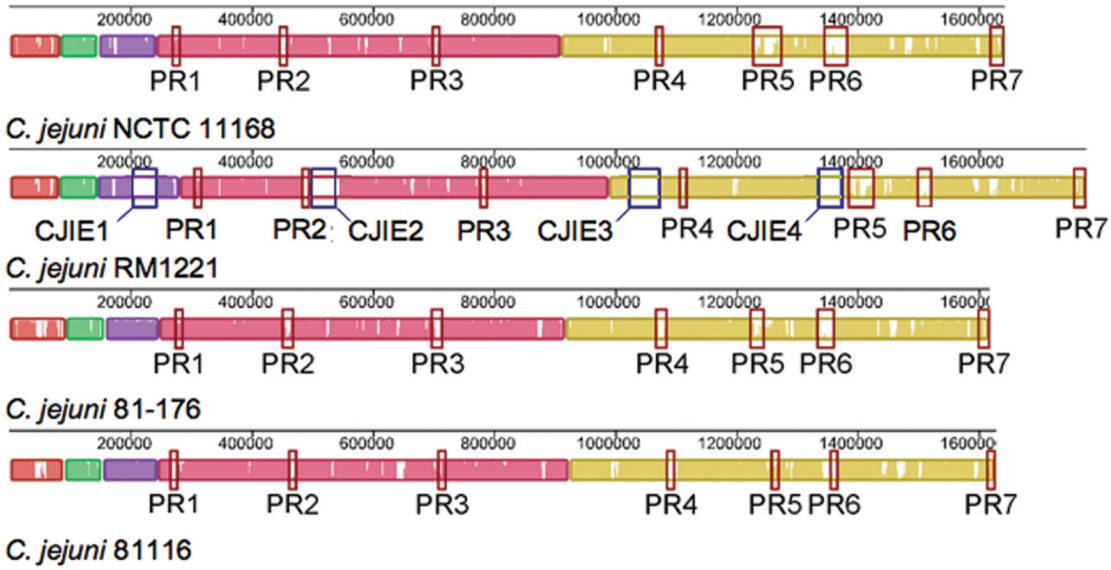

Figure 1.

Whole genome alignments of *C. jejuni* NCTC 11168, RM1221, 81−176 and 81116 performed using Mauve [42]. Each genome is laid out horizontally and homologous segments are shown as colored blocks. The average sequence identity is proportional to the height of the colored region with in each horizontal block. *C. jejuni* integrated elements (CJIEs) in strain RM1221 are indicated by blue rectangles. The seven plasticity regions (PR1-PR7) are indicated by red rectangles.

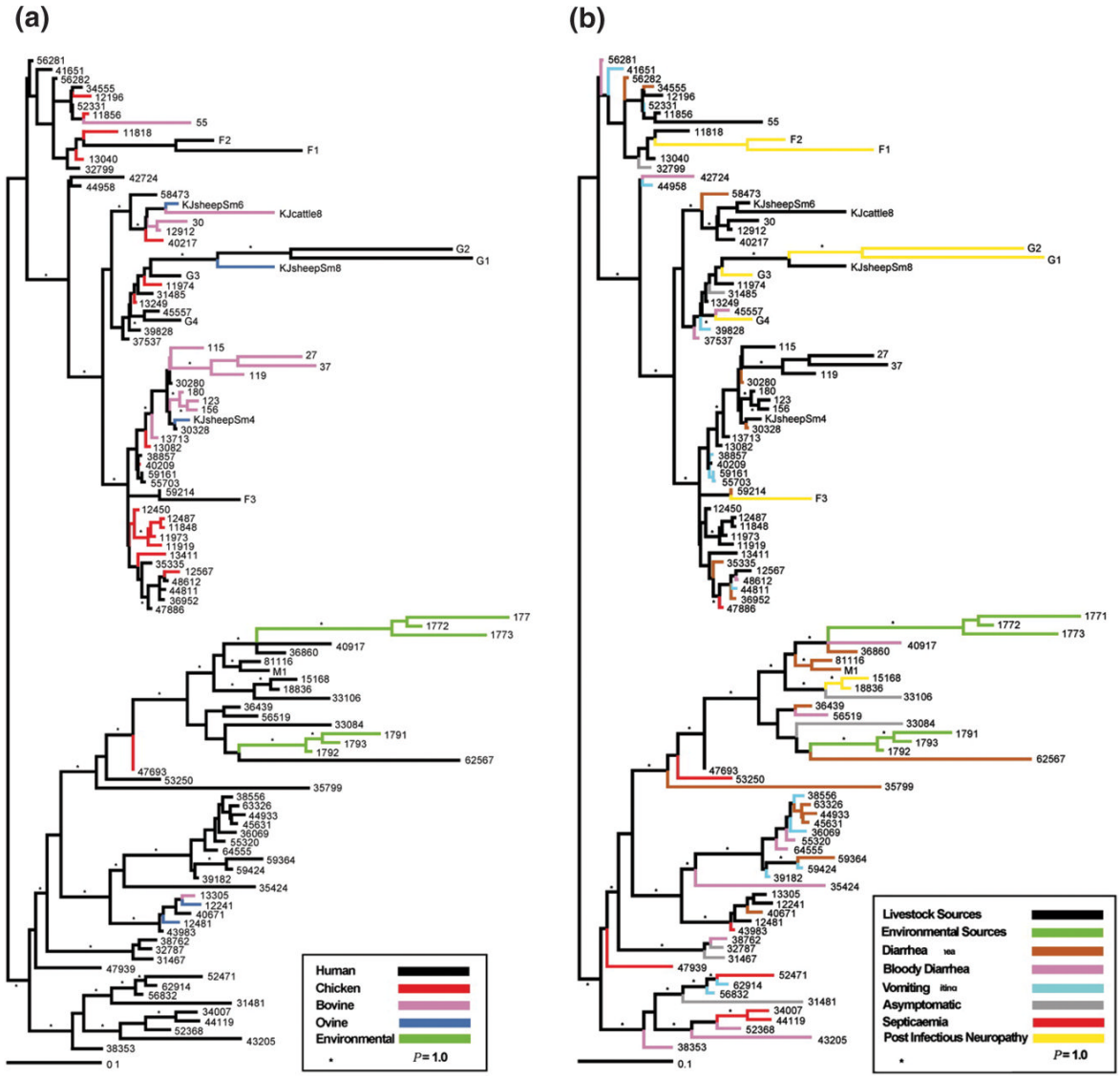

Figure 2.

Phylogenomic relationship of *C. jejuni* strains. Strains are designated at the end of branches and are colored according to the **(A)** ecological niche from which the *C. jejuni* strain was isolated or **(B)** clinical symptoms or livestock/environmental source. P = 1.0 represents 100% of all phylogenies showing a given topology. Adapted with permission from the authors [20].

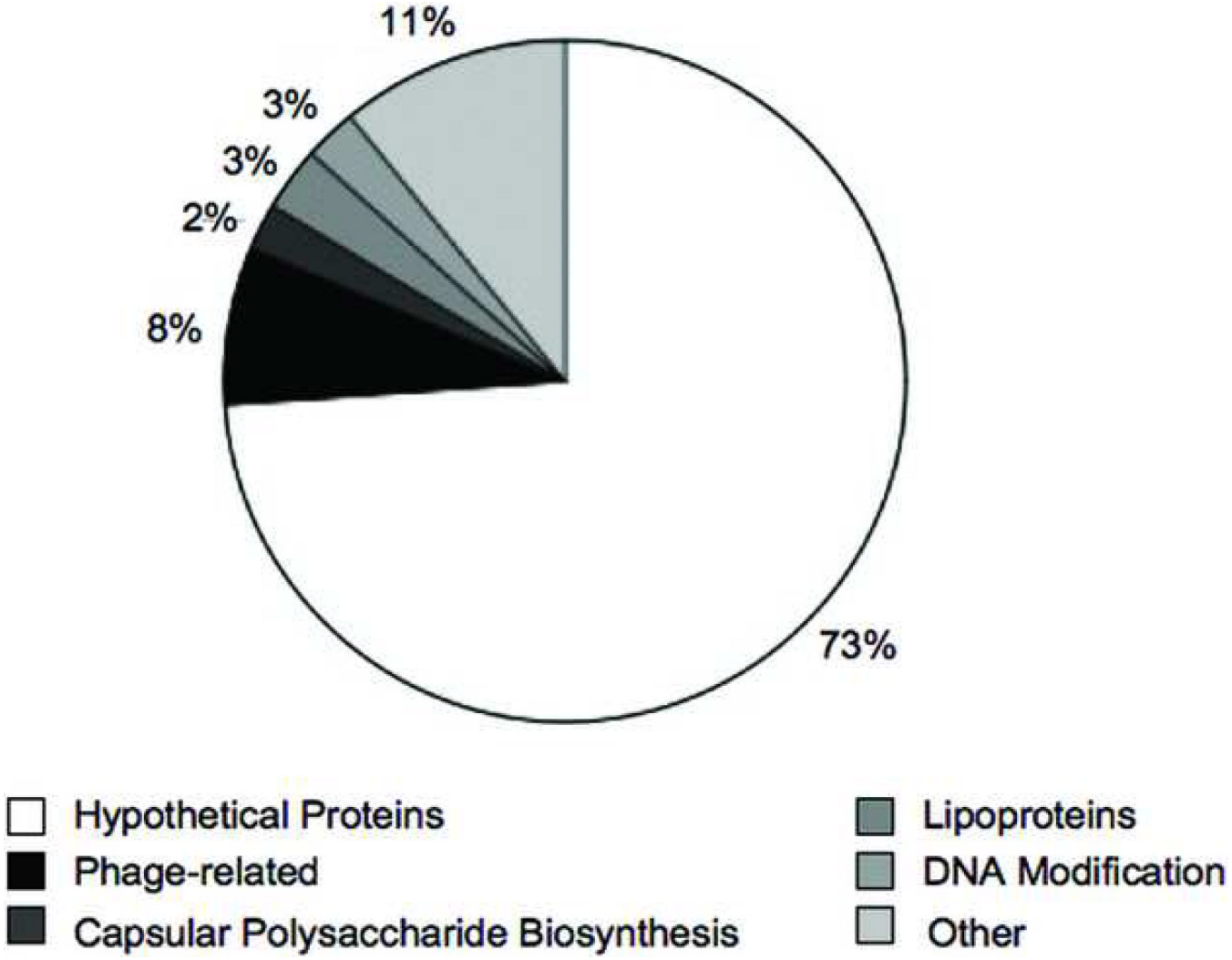

Figure 3.
Classification of genes unique to one of the four *C. jejuni* strains (NCTC 11168, RM1221, 81−176 and 81116). Unique genes are listed in Supplemental Table 1 and were classified based upon genome annotations

Table 1

Features of Sequenced *Campylobacter* Genomes

Species/Strain	Size (Mb)	%GC	ORFs	Origin	Disease[a]	GenBank[b]	Ref.
Campylobacter jejuni subsp *jejuni* NCTC 11168	1.64	30.5	1643	Clinical	Food Poisoning	AL111168	[36]
Campylobacter jejuni subsp *jejuni* RM1221	1.78	30.3	1835	Chicken		CP000025	[22]
Campylobacter jejuni subsp *jejuni* 81−176	1.6	30.6	1653	Clinical	Food Poisoning	CP000538	[24]
Campylobacter jejuni subsp *jejuni* 81116	1.63	30.5	1626	Clinical	Food Poisoning	CP000814	[43]
Campylobacter jejuni subsp *jejuni* CG8421	1.6	30.4	1512	Clinical	Food Poisoning	ABGQ00000000	
Campylobacter jejuni subsp *jejuni* HB93−13	1.7	30.6	1710	Clinical	GBS[c]	AANQ00000000	
Campylobacter jejuni subsp *jejuni* CG8486	1.65	30.4	1425	Clinical	Food Poisoning	AASY00000000	[44]
Campylobacter jejuni subsp *jejuni* CF93−6	1.67	30.5	1757	Clinical	MFS[d]	AANJ00000000	
Campylobacter jejuni subsp *jejuni* 84−25	1.67	30.4	1748	Clinical	Meningitis	AANT00000000	
Campylobacter jejuni subsp *jejuni* 260.94	1.65	30.5	1716	Clinical	GBS	AANK00000000	
Campylobacter jejuni subsp *doylei* 269.97	1.8	30.6	1731	Clinical	Bacteremia	CP000768	
Campylobacter coli RM2228	1.68	31.4	1765	Chicken			[22]
Campylobacter concisus 13826	2.1	39.4	1929	Clinical	Food Poisoning	CP000792	
Campylobacter curvus 525.92	2.0	44.5	1931	Clinical	Periodontitis	CP000767	
Campylobacter fetus subsp. *fetus* 82−40	1.8	33.3	1719	Clinical	Septicemia	CP000487	
Campylobacter hominis ATCC BAA-381	1.7	31.7	1682	Clinical	Non-pathogenic	CP000776	
Campylobacter lari RM2100	1.5	29.6	1554	Clinical	Food Poisoning		[22]
Campylobacter upsaliensis RM3195	1.66	34.5	1782	Clinical	GBS		[22]

[a] Disease associated with origin of isolate

[b] GenBank Accession Number

[c] Guillain-Barre Syndrome

[d] Miller-Fisher Syndrome