Genomic characterization of *Campylobacter jejuni* associated with perimyocarditis: a family case report

Irene Ortega-Sanz[a], Marcial García[a], Carolina Bocigas[a], Gregoria Megías[b], Beatriz Melero[a*], Jordi Rovira[a*]

[a]Department of Biotechnology and Food Science, University of Burgos, Burgos, Spain

[b]Microbiology Department of the University Hospital of Burgos (HUBU), Burgos, Spain

Running headline: perimyocarditis-related *C. jejuni*

[*]Corresponding author: Jordi Rovira[a], jrovira@ubu.es

Beatriz Melero[a], bmelero@ubu.es

[a]Department of Biotechnology and Food Science, University of Burgos, Burgos, Spain

Keywords: campylobacteriosis, perimyocarditis, clinical case, phase-variation, virulence genes, antimicrobial resistance genes

Abstract

Campylobacter spp. is the leading cause of foodborne gastrointestinal infections in humans worldwide. We report the first case of a family that had contact with the same source of *C. jejuni* contamination with different results, and the first case of the youngest patient affected by *C. jejuni*-related perimyocarditis published to date. Only the little siblings were infected by the same *C. jejuni* strain, but with different symptoms. The daughter was slightly affected with mild enteritis and the son suffered a longer campylobacteriosis followed with a perimyocarditis. Both strains were sequenced for their genomic characterization and compared to *C. jejuni* NCTC 11168 genome in order to get insights of the causes that may be related to perimyocarditis. Various comparison tools were used for the comparative genomics analysis, including the identification of virulence and antimicrobial resistance genes, and phase-variable (PV) genes. The strains suffered little but significant genomic changes affecting the ON/OFF state of PV genes after passing through both hosts, indicating that PV occurs during human colonization, which modulates bacteria virulence, through human host adaptation, that ultimately is related to complications following a campylobacteriosis episode depending on the host status. The findings are discussed in the background of the perimyocarditis case and highlight the importance of the relation between host and pathogen in severe complications of *Campylobacter* infections.

1. Introduction

Campylobacteriosis is a foodborne illness caused by bacteria of the genus *Campylobacter*, mainly *C. jejuni* (83.9 %), *C. coli* (10.3 %) and *C. lari* (0.1 %). It is considered the number-one worldwide diarrheal disease caused by bacteria and the most frequently reported foodborne illness in the EU since 2005 with more than 220,000 cases in 2019 (EFSA and ECDC, 2021), but the COVID-19 pandemic and the withdrawal of the UK from the EU reduced the number of reported cases to approximately 127,000 in 2021 (EFSA and ECDC, 2022). EFSA estimated the cost of campylobacteriosis to EU public health systems to be around 2.4 billion euros per year (EFSA, 2011). *Campylobacter* spp. is ubiquitous and lives in the intestinal tract of healthy poultry, pigs and cattle, but it may also be found in the feces (Kaakoush *et al*, 2015). The common routes of infection are consumption of raw, undercooked or poorly handled poultry meat that has been contaminated during slaughtering, unpasteurized milk and untreated water. Symptoms tend to arise around two to five days after exposure to the bacteria and include fever, nausea and vomits leading to abdominal pain and cramps and diarrhea that may be bloody. Although campylobacteriosis is usually a self-limiting illness and lasts about one week without antibiotic treatment, the use of antibiotics recommended for severe cases has led to the development of antibiotic-resistant *Campylobacter* strains that limit drugs usefulness (Kaakoush *et al*, 2015). As a consequence, *Campylobacter* infections could become a public health concern.

Despite post-infection complications are rare, they can occur mainly in young children, elderly and immunocompromised people including chronic inflammatory diseases, such as Guillain–Barré syndrome (GBS) and Miller Fischer syndrome

(MF), reactive arthritis, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (Louwen and Hays, 2013). GBS and MF are autoimmune disorders in which the peripheral nerves are damaged causing muscle weakness and sometimes paralysis (Koga *et al*, 2005; van Doorn *et al*, 2008). The mechanism underlying these diseases is known as molecular mimicry between *Campylobacter* lipooligosaccharides (LOS) and peripheral nerve gangliosides, which produces a cross-reaction where antibodies attack the human nerves. The molecular mimicry is mainly associated with *C. jejuni*, but also with other *Campylobacter* species, particularly with *C. coli* (Bersudsky *et al*, 2000; Koga *et al*, 2005). However, the role of *Campylobacter* in human inflammatory diseases is frequently underestimated due to speedy recovery from infection.

Another important complication that has been associated with *Campylobacter* infection is perimyocarditis (Louwen and Hays, 2013). Myocarditis is the inflammation of the heart muscle that originates chest pain, palpitations or breathlessness and may result in heart failure of the patient and sudden death. Electrocardiogram (ECG) changes and elevated troponin levels follow the inflammation (Spapen *et al*, 2015). In the last years, up to 53 publications were found in the PubMed database (consulted in September 2022) showing *Campylobacter* spp. as the trigger agent of myocarditis or perimyocarditis from a clinical point of view. However, none of them sequenced or characterized the bacterial genome. Thus, the mechanism underlying this complication is still unknown. Some theories propose a direct cardiac invasion by the bacteria or its toxins or an immune-mediated inflammation (Spapen *et al*, 2015). What is clearly revealed is that among all the reported cases, young males are more likely to suffer from this disease (Florkowski *et al*, 1984; Cox *et al*, 2001; Hannu *et al*,

2005; Pena and Fishbein, 2007; Turley *et al*, 2008; Hull and Varma, 2011; Panikkath *et al*, 2014). Nonetheless, the high impact of campylobacteriosis on public health systems suggests that *C. jejuni*-related perimyocarditis and other post-infection complications could be underreported. There is evidence of prolonged and asymptomatic *Campylobacter* infections among children especially in low- and middle-income countries that are underdiagnosed and underreported (Lee et al, 2013).

In this work, it is described the first familiar case that had contact with the same source of *C. jejuni* contamination, in which only the little siblings were infected by *C. jejuni* and only one of them suffered from perimyocarditis. The strains isolated from the children were molecularly typed and sequenced to study the genomic traits that may be related to this complication, and were compared with the complete reference *C. jejuni* subsp. *jejuni* NCTC 11168 publicly available genome.

2. Clinical case report

An 11-year-old male with no relevant medical history was attended by the physician due to a severe liquid and bloody diarrhea event the second day after eating chicken wings with his family. Also, his 13-year-old sister suffered from this event, although she recovered in five days. At that moment, a stool culture confirmed the medical diagnosis of *C. jejuni* infection in both patients. No rotavirus, adenovirus or *Clostridioides difficile* were found in any of these samples. Two days later, the male went to the hospital emergency room complaining about an acute and severe retrosternal chest pain radiating the throat, although cardiac examination was normal. However, electrocardiogram

revealed ST-segment elevation and a myocardial injury was confirmed due to the high concentration of cardiac troponin (hscTn), that was 108 ng L^{-1} compared to the cut-off of 22 ng L^{-1} for men approved by FDA (2017). The patient was finally diagnosed with a non-ischemic perimyocarditis triggered by *C. jejuni*, and after antibiotic therapy he was discharged from the hospital free of complaints seven days since hospitalization.

3. Materials and Methods

Two *C. jejuni* strains (H660, girl; H661, boy) were isolated from stool samples by the University Hospital of Burgos (Spain). *C. jejuni* isolates were stored at -80 ºC in cryovials containing Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, England) supplemented with 20 % glycerol until use. Prior to use in assays, *C. jejuni* isolates were cultured on Nutrient blood agar (BA) prepared from Broth No. 2 (Oxoid) containing 1.5 % bacteriological agar (Oxoid) supplemented with 5 % (v v^{-1}) defibrinated sheep blood (Oxoid) under microaerobic conditions (48 h at 41.5 ºC).

3.1.1. Pulsed Field Gel Electrophoresis (PFGE)

For bacterial typing, PFGE analyses were based on the CDC's PulseNet protocol (https://www.cdc.gov/pulsenet/pathogenes/pfge.html).

3.1.2. DNA Extraction

A single colony of each isolate was recovered from the BA agar in BHI broth and incubated at 37 ºC overnight in a microaerobic atmosphere. Bacterial DNA was extracted using the commercial QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions and purity was determined

using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

3.1.3. Sequencing and *in silico* Analysis

Library preparation and sequencing was performed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and the MiSeq platform (Illumina) providing 2 × 300 bp paired-end reads. The in-house pipeline CamPype (Barbero JA, https://github.com/JoseBarbero/CamPype) was followed for the bioinformatics analysis (Supplemental File 1).

4. Results

4.1. Genome Characterization of *C. jejuni* H660 and H661

Complete information on the characteristics of the genomic sequences of the two isolates (H660 and H661) and the reference strain NCTC 11168 is summarized in Table 2. Briefly, the completeness of the assembled genomes, H660 and H661, was equal to the complete NCTC 11168 reference genome (99.7 %). Moreover, H660 and H661 had a slightly larger genome compared to NCTC 11168, with a greater number of coding DNA sequences (CDS), mainly hypothetical proteins, although a minor G+C content. Coding-DNA sequences were annotated proportionally to the genome length, in which H660 genome was 914 bp larger than H661 genome, and, therefore, three more genes were annotated in H660 compared to H661. The genetic relatedness among the two isolates and the reference genome resulted in ANI values greater than 99 % (and > 99.99 % between H660 and H661). Genotypically, the three *C. jejuni* strains were grouped together in the same clonal complex CC21, although H660 and H661 were

clustered into the same PFGE type and Sequence Type (ST)-148, and NCTC 11168 corresponded to ST-43.

4.2. Virulence Genes

The presence/absence of 76 virulence genes (Table 1) associated with the pathogenicity of *C. jejuni* was evaluated in H660, H661 and NCTC 11168 strains. The virulence pattern of H660 and H661 was equal to the reference *C. jejuni* NCTC 11168. The presence of *cst-III* in the two isolates is associated to LOS locus class C, matching NCTC 11168. *VirB11*, *ggt*, *cgtB*, *cst-II* and the Type VI Secretion System (T6SS) coding genes were not present in any of the three strains.

4.3. Antimicrobial Resistance (AMR) Genes

ABRicate included all three CmeABC efflux pump genes (*cmeA*, *cmeB* and *cmeC*) along with the *cmeR* repressor in all the three strains tested, whereas AMRFinder identified the $bla_{OXA\text{-}193}$ gen (resistance to β-lactam) in the three strains, and *tet(O)* (resistance to tetracyclines) exclusively in *C. jejuni* H660 and H661. In addition, H660 and H661 genomes also harbored T86I substitution in GyrA (resistance to (fluoro)quinolones). Other unknown mutations (not biologically evaluated yet) were also observed in both isolated strains. These were R285K in GyrA and 296C>G, 298G>A, 327G>A, 364G>C, 554A>C, 571T>G, 1027A>G, 1752T>C in *23S rRNA*.

4.4. Pan-genome Analysis

A total of 1,900 gene clusters were identified in the pan-genome including H660, H661 and NCTC 11168 genomes. The core-genome consisted of 1,478 genes that were present in all strains, and the accessory-genome was composed of 422

189 genes, including exclusive genes to each strain and those genes present in just
190 two genomes. Although the greatest number of differences was observed with
191 respect to the NCTC 11168 reference strain, interestingly, some important
192 differences were observed between the perimyocarditis-related strain (H661) and
193 the non- perimyocarditis-inducing strain (H660). Only two genes were exclusive
194 to H660, both of them coding hypothetical proteins whose function could not be
195 assigned neither by NCBI database nor UniProtKB, whereas just one was
196 exclusively present in H661 coding a putative methyl-accepting chemotaxis
197 signal transduction protein. The remaining differences corresponded to phase-
198 variable (PV) genes, mainly involved in the flagella locus, or fragmented genes
199 at a contig boundary, indicating that were more likely to have been split by an
200 assembly issue.

201 **4.5. Phase-variable (PV) Genes**

202 The analysis of PV genes revealed the existence of 36 homology groups, from
203 which six were exclusive to NCTC 11168, two to H660, one to H661 and two to
204 both H660 and H661. Although there were 29 polyG/polyC tracts of seven or
205 more repeats in *C. jejuni* NCTC11168, the sequencing allowed the identification
206 of 26 polyG/C tracts in both *C. jejuni* H660 and H661. From these, 21 were
207 located within coding regions (intragenic tracts) in strain H660, two more
208 compared to H661, whereas the rest were close to ORFs (intergenic tracts).
209 Table 3 summarizes the PV tract differences identified between H660 and H661
210 and shows that the ON/OFF state of seven genes (*kdpB*, *flgR*, *wlaN*, *cj1144c*,
211 *cj1305c*, *cj1310c* and *cj1420c*) varied in both isolates.

212 **5. Discussion**

The diagnosis of non-ischemic perimyocarditis caused by *C. jejuni* was established by a combination of clinical and laboratory analysis. In general, enterovirus and adenovirus infections are the most frequent cause of myocarditis, but the awareness of *C. jejuni*-associated perimyocarditis has increased since the first reported case in 1980 by Pönkä *et al* (1980). According to this work, subsequent research studies on *C. jejuni*-associated perimyocarditis have showed that immunocompetent young males are more susceptible to infection, accounting for more than 90 % of cases, which could be due to the influence of sex hormones on cardiac function (Hessulf *et al*, 2016). Testosterone was shown to prevent anti-inflammatory responses in male mice, whereas estrogen demonstrated to protect against viral infectivity in the cardiac muscle cells of female mice (Lyden *et al*, 1987; Ang *et al*, 2001). Thus, gender has a great impact on perimyocarditis development as it is highlighted in this clinical case.

The equivalent completeness of the two human *C. jejuni* H660 (girl) and H661 (boy) isolate assemblies compared to the reference NCTC 11168 ensured their confidence for comparative genome analyses despite being fragmented in contigs. The two isolates (H660 and H661) were clustered into the same PFGE type, ST-148 and CC21, and showed high ANI value, demonstrating high similarities between them and that both isolates corresponded to the same *C. jejuni* strain with identical genotypes. The PubMLST database (accessed in December 2022) (Jolley *et al*, 2018) showed that CC21 was the most representative lineage with 18,588 isolates from a total of 103,553 submitted isolates (18 %). In Spain, this clonal complex is the second largest found to date in the database with 45 out of 479 isolates (9 %). Of note, CC21 has been frequently related to episodes of human campylobacteriosis and has been

isolated from a wide range of food chain sources (Dingle *et al*, 2001). Furthermore, although specific *Campylobacter* genotypes have been shown to be potentially linked to increased risk of post-infection complications, in particular for irritable bowel syndrome (PI-IBS), such complication developed in just over half (55%) of cases associated to CC21 isolates (Peters *et al*, 2021). Thus, the potential association of this common lineage with the perimyocarditis triggered by one of the two isolates (H661) (50 %) reveals the importance of characterizing their genomes looking for specific characteristics promoting perimyocarditis development, as previously did Peters *et al* (2021) for PI-IBS.

The mechanisms of pathogenicity of *C. jejuni* include four main steps: adhesion to intestinal cells, colonization of the digestive tract, invasion of targeted cells and toxin production (Haddad *et al*, 2010). As numerous *Campylobacter* genes have been previously described to be involved in the different stages (Bolton, 2015), a database with 63 of them and the 13 genes of the Type VI secretion system T6SS (*tssA-tssM*) was built to be used as the query for tBLASTn. No differences were found between H660, H661 and NCTC 11168 genomes among the 76 virulence genes selected, indicating that the virulence of these human isolates might be similar to that of NCTC 11168. These results were comparable to another study reporting presence/absence of virulence genes in CC21, with the exception of *cfrB* (García-Sánchez *et al*, 2019), a ferric enterobactin receptor that was truncated in H660, H661 and NCTC 11168 (*cj0444*), which has been related to reduced growth promotion of bacteria under iron-limited conditions and colonization of the intestines (Xu *et al*, 2010). However, the ability of *Campylobacter* to colonize the chicken gut does not extensively rely on the virulence factor profile, but rather on environmental factors, e.g., nutrient

availability, osmolarity, pH and organic acid concentrations, and its capability to translocate across the intestinal barrier (Awad *et al*, 2016), which may be influenced by nucleotide differences (ON/OFF state) in these virulence genes (Duong and Konkel, 2009). Thus, it seems clear that there is a need not only to look for differences in virulence genes between strains, but also to analyse possible variations in the sequences of these genes.

The pan-genome analysis of H660 and H661 revealed the existence of certain differences between both strains, most of which were observed in PV genes. Phase variation is a common phenomenon during replication for niche adaption and survival influencing host colonization (Cayrou *et al*, 2021). Reversible mutations in simple sequence repeats (SSR) of these PV genes seem to alter their gene expression and could affect the pathogenicity of *C. jejuni* strains. The presence of hypervariable genes together with the absence of a functional DNA mismatch repair system in the bacteria promote diversity among the population faster and increase adaptability of bacteria to new environments. Variation in the ON/OFF state in the PV genes has been observed to occur during colonization in chickens and mouse infection models, and even during bacterial survival against bacteriophages. PV genes are mainly polyG/C repeat tracts and often associated with the biosynthesis or modification of surface structures, flagellar modification and unknown function (Linton *et al*, 2000; Parkhill *et al*, 2000; Ashgar *et al*, 2007). Comparing the annotated genes with PV tracts that showed differences between H660 and H661 (Table 3), the strain H661 had in total one more PV gen in the ON state and one less in the OFF state, but one intergenic homopolymeric tract in the upstream region of *cj1144c* that might affect transcription of the hypothetical protein Cj1144c. As a consequence, the

functionalities of both H660 and H661 strains could be different, and some of these genes activated/deactivated in H660 or H661 (*kdpB*, *flgR*, *wlaN*, *cj1144c*, *cj1305c*, *cj1310c* and *cj1420c*) could play a role in host adapatation and subsequent consequences, as only the boy suffered from a perimyocarditis.

The gen *kdpB* encodes a potassium-transporting ATPase ATP-binding subunit that is responsible for the energy generation in the Kdp system (Durell *et al*, 2000) under low-potassium conditions to maintain constant turgor pressure within the cell (Hofreuter *et al*, 2006). It was described that low-potassium level (hypokalemia) is the result of episodes of diarrhea and vomits, and could cause cardiac rhythm disturbances (Skogestad and Aronsen, 2018). Hence, the ON state of *kdpB* in H661 could enhance the prevalence of bacteria under adverse potassium conditions in the host and thus extending the infection in the patient.

The gen *wlaN* encodes a β-1,3-galactosyltransferase enzyme that is required for the production of GM1-like sialylated lipooligosaccharide (LOS^{SIAL}) from GM2-like structure. In H660 and NCTC 11168, *wlaN* presented an homopolimeric tract of 8G able to synthesize a GM1-like structure (ON state), whereas in H661, *wlaN* seemed to code a truncated protein keeping the GM2-like structure (OFF state) (Linton *et al*, 2000; Gilbert *et al*, 2002; Guirado *et al*, 2020). LOS^{SIAL} is related to the virulence of the bacteria, increasing epithelial invasiveness (Louwen *et al*, 2008) and protection against complement-mediated killing (Guerry *et al*, 2000), but this structure could trigger an anti-inflammatory reaction suggesting that the bacteria might be indirectly involved in the development of perimyocarditis and so the immune system could influence it (Hameed *et al*, 2020).

Other important differences between H660 and H661 genomes were observed in the flagella o-linked glycosylation loci (*cj1293-cj1342c*). H660 showed an homopolimeric tract of 7A in *flgR* coding a truncated protein. This *flgR* gen is very important for motility as it is a transcriptional regulator of flagellar function and activates sigma54-dependent flagellar genes in *C. jejuni*. In fact, it has been previously observed that *flgR* mutants were nonflagellate (Jagannathan *et al*, 2001), which could affect the pathogenic potential of *C. jejuni* isolates (Malik-Kale *et al*, 2007). Thus, this potential absence of motility in H660 related to the truncated FlgR, which must be experimentally evaluated, could affect the adhesion and invasion capacity of the strain to intestinal cells. Moreover, Cj1310c (uncharacterized protein) that was duplicated in the strain H661 and in the ON state might assist in the colonization and adherence to host cells (Sher *et al*, 2020), increasing its pathogenicity compared to H660. These data suggest that, despite both isolates corresponded to the same *C. jejuni* strain, the different status of the PV genes associated to chemotaxis, LOS and flagella locus reveals that phase variation occurs even during human colonization turning the bacteria into a more or less virulence strain depending on host environment, which could have some influence in the duration and severity of the campylobacteriosis, and further complications including perimyocarditis according to human host status.

6. Conclusions

The extremely high similarity between the two isolated *C. jejuni* strains make difficult to draw conclusions in the genomic features that could be linked to perimyocarditis. However, the different PV traits found in both isolates demonstrate that phase variation in *C. jejuni* occurs during human colonization modulating bacteria virulence, through human host adaptation, that ultimately is

related to complications following a campylobacteriosis episode depending on the host status. This suggests the importance of the relation between host and pathogen in severe complications of *Campylobacter* infections. To help diagnose *Campylobacter* complications in patients with suspected perimyocarditis, it could be useful to include this bacterium in the serological test specially when this episode follows a campylobacteriosis. Future studies will be performed to better characterize both isolates and elucidate the pathogenesis of this bacteria, including identification of SNPs and phenotypic studies, such as motility test and *in vitro* cell adhesion and invasion assays.

7. Author Contribution Statement

Irene Ortega-Sanz: conceptualization, investigation, data curation, formal analysis, methodology, software, visualization, writing – original draft, writing – review & editing. **Marcial García**: data curation, writing – review & editing. **Carolina Bocigas**: methodology. **Gregoria Megías**: resources. **Beatriz Melero**: funding acquisition, project administration, resources, writing – review & editing. **Jordi Rovira**: conceptualization, funding acquisition, project supervision, writing – review & editing.

8. Conflict Of Interest

No conflict of interest declared.

9. Funding statement

The project leading to these results has received funding from “La Caixa” Foundation and Caja Burgos Foundation, under agreement LCF/PR/PR18/51130007. Ortega-Sanz I received a predoctoral grant from the

Junta of Castile and León, cofinanced by the Ministry of Education of the Government of Castile and León and the European Social Fund.

10. References

Aidley J, Wanford JJ, Green LR, et al. PhasomeIt: an 'omics' approach to cataloguing the potential breadth of phase variation in the genus Campylobacter. Microb Genom 2018;4(11):e000228. doi: 10.1099/mgen.0.000228.

Ang CW, De Klerk MA, Endtz HP, et al. Guillain-Barré syndrome- and Miller Fisher syndrome-associated Campylobacter jejuni lipopolysaccharides induce anti-GM1 and anti-GQ1b Antibodies in rabbits. Infect Immun 2001;69(4):2462-9. doi: 10.1128/IAI.69.4.2462-2469.2001.

Ashgar SS, Oldfield NJ, Wooldridge KG, et al. CapA, an autotransporter protein of Campylobacter jejuni, mediates association with human epithelial cells and colonization of the chicken gut. J Bacteriol 2007;189(5):1856-65. doi: 10.1128/JB.01427-06.

Awad WA, Dublecz F, Hess C, et al. Campylobacter jejuni colonization promotes the translocation of Escherichia coli to extra-intestinal organs and disturbs the short-chain fatty acids profiles in the chicken gut. Poult Sci 2016;95(10):2259-65. doi: 10.3382/ps/pew151.

Bersudsky M, Rosenberg P, Rudensky B, et al. Lipopolysaccharides of a Campylobacter coli isolate from a patient with Guillain-Barré syndrome display ganglioside mimicry. Neuromuscul Disord 2000;10(3):182-6. doi: 10.1016/s0960-8966(99)00106-6.

382 Bolton DJ (2015) Campylobacter virulence and survival factors. Food Microbiol
383 2015;48:99-108. doi: 10.1016/j.fm.2014.11.017.

384 Cayrou C, Barratt NA, Ketley JM, et al. Phase Variation During Host Colonization
385 and Invasion by Campylobacter jejuni and Other Campylobacter Species.
386 Front Microbiol 2021;12:705139. doi: 10.3389/fmicb.2021.705139.

387 Cox ID, Fluck DS, Joy MD. Campylobacter myocarditis; loose bowels and a
388 baggy heart. Eur J Heart Fail 2001;3(1):105-7. doi: 10.1016/s1388-
389 9842(00)00093-3.

390 Dingle KE, Colles FM, Wareing DR, et al. Multilocus sequence typing system for
391 Campylobacter jejuni. J Clin Microbiol 2001;39(1):14-23. doi:
392 10.1128/JCM.39.1.14-23.2001.

393 Duong T, Konkel ME. Comparative studies of Campylobacter jejuni genomic
394 diversity reveal the importance of core and dispensable genes in the biology
395 of this enigmatic food-borne pathogen. Curr Opin Biotechnol 2009;20(2):158-
396 65. doi: 10.1016/j.copbio.2009.03.004.

397 Durell SR, Bakker EP, Guy HR. Does the KdpA subunit from the high affinity K(+)-
398 translocating P-type KDP-ATPase have a structure similar to that of K(+)
399 channels? Biophys J 2000;78(1):188-99. doi: 10.1016/S0006-
400 3495(00)76584-2.

401 EFSA. Scientific Opinion on Campylobacter in broiler meat production: control
402 options and performance objectives and/or targets at different stages of the
403 food chain. EFSA Journal 2011;9(4):2105. doi: 10.2903/j.efsa.2011.2105.

EFSA and ECDC. The European Union One Health 2019 Zoonoses Report. EFSA Journal 2021;19(2):6406. doi: 10.2903/j.efsa.2021.6406.

EFSA and ECDC (2022) The European Union One Health 2021 Zoonoses Report. EFSA Journal 2022;20(12):7666. doi: 10.2903/j.efsa.2022.7666

FDA 510(k) Substantial Equivalence Determination Decision Summary Assay Only Template. 2017. Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K162895.pdf [Last accessed: 01/30/2023]

Florkowski CM, Ikram RB, Crozier IM, et al. Campylobacter jejuni myocarditis. Clin Cardiol 1984;7(10):558. doi: 10.1002/clc.4960071008.

García-Sánchez L, Melero B, Jaime I, et al. Biofilm formation, virulence and antimicrobial resistance of different Campylobacter jejuni isolates from a poultry slaughterhouse. Food Microbiol 2019;83:193-199. doi: 10.1016/j.fm.2019.05.016.

Gilbert M, Karwaski MF, Bernatchez S, et al. The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, Campylobacter jejuni. Biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. J Biol Chem 2002;277(1):327-37. doi: 10.1074/jbc.M108452200.

Guerry P, Ewing CP, Hickey TE, et al. Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of Campylobacter jejuni. Infect Immun 2000;68(12):6656-62. doi: 10.1128/IAI.68.12.6656-6662.2000.

Guirado P, Paytubi S, Miró E, et al. Differential Distribution of the wlaN and cgtB Genes, Associated with Guillain-Barré Syndrome, in Campylobacter jejuni

427 Isolates from Humans, Broiler Chickens, and Wild Birds. Microorganisms
428 2020;8(3):325. doi: 10.3390/microorganisms8030325.

429 Haddad N, Marce C, Magras C, et al. An overview of methods used to clarify
430 pathogenesis mechanisms of Campylobacter jejuni. J Food Prot
431 2010;73(4):786-802. doi: 10.4315/0362-028x-73.4.786.

432 Hameed A, Woodacre A, Machado LR, et al. An Updated Classification System
433 and Review of the Lipooligosaccharide Biosynthesis Gene Locus in
434 Campylobacter jejuni. Front Microbiol 2020;11:677. doi:
435 10.3389/fmicb.2020.00677.

436 Hannu T, Mattila L, Rautelin H, et al. Three cases of cardiac complications
437 associated with Campylobacter jejuni infection and review of the literature.
438 Eur J Clin Microbiol Infect Dis 2005;24(9):619-22. doi: 10.1007/s10096-005-
439 0001-2.

440 Hessulf F, Ljungberg J, Johansson PA, et al. Campylobacter jejuni-associated
441 perimyocarditis: two case reports and review of the literature. BMC Infect Dis
442 2016;16:289. doi: 10.1186/s12879-016-1635-7.

443 Hofreuter D, Tsai J, Watson RO, et al. Unique features of a highly pathogenic
444 Campylobacter jejuni strain. Infect Immun 2006;74(8):4694-707. doi:
445 10.1128/IAI.00210-06.

446 Hull SR, Varma MP. Myopericarditis following Campylobacter infection. Ir J Med
447 Sci 2011;180(3):753-5. doi: 10.1007/s11845-009-0314-8.

448 Jagannathan A, Constantinidou C, Penn CW. Roles of rpoN, fliA, and flgR in
449 expression of flagella in Campylobacter jejuni. J Bacteriol 2001;183(9):2937-
450 42. doi: 10.1128/JB.183.9.2937-2942.2001.

451 Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics:
452 BIGSdb software, the PubMLST.org website and their applications.
453 Wellcome Open Res 2018;3:124. doi: 10.12688/wellcomeopenres.14826.1.

454 Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, et al. Global Epidemiology of
455 Campylobacter Infection. Clin Microbiol Rev 2015;28(3):687-720. doi:
456 10.1128/CMR.00006-15.

457 Koga M, Gilbert M, Li J, et al. Antecedent infections in Fisher syndrome: a
458 common pathogenesis of molecular mimicry. Neurology 2005;64(9):1605-11.
459 doi: 10.1212/01.WNL.0000160399.08456.7C.

460 Lee G, Pan W, Peñataro Yori P, et al. Symptomatic and asymptomatic
461 Campylobacter infections associated with reduced growth in Peruvian
462 children. PLoS Negl Trop Dis 2013;7(1):e2036. doi:
463 10.1371/journal.pntd.0002036.

464 Linton D, Gilbert M, Hitchen PG, et al. Phase variation of a beta-1,3
465 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-
466 oligosaccharide of Campylobacter jejuni. Mol Microbiol 2000;37(3):501-14.
467 doi: 10.1046/j.1365-2958.2000.02020.x.

468 Louwen R, Heikema A, van Belkum A, et al. The sialylated lipooligosaccharide
469 outer core in Campylobacter jejuni is an important determinant for epithelial
470 cell invasion. Infect Immun 2008;76(10):4431-8. doi: 10.1128/IAI.00321-08.

471 Louwen R, Hays J. Is there an unrecognised role for Campylobacter infections in
472 (chronic) inflammatory diseases? World J Clin Infect Dis 2013;3(4):58. doi:
473 10.5495/wjcid.v3.i4.58.

474 Lyden DC, Olszewski J, Feran M, et al. Coxsackievirus B-3-induced myocarditis.
475 Effect of sex steroids on viremia and infectivity of cardiocytes. Am J Pathol
476 1987;126(3):432-8.

477 Malik-Kale P, Raphael BH, Parker CT, et al. Characterization of genetically
478 matched isolates of Campylobacter jejuni reveals that mutations in genes
479 involved in flagellar biosynthesis alter the organism's virulence potential.
480 Microbiol 2007;73(10):3123-36. doi: 10.1128/AEM.01399-06.

481 Panikkath R, Costilla V, Hoang P, et al. Chest pain and diarrhea: a case of
482 Campylobacter jejuni-associated myocarditis. J Emerg Med 2014;46(2):180-
483 3. doi: 10.1016/j.jemermed.2013.08.060.

484 Parkhill J, Wren BW, Mungall K, et al. The genome sequence of the food-borne
485 pathogen Campylobacter jejuni reveals hypervariable sequences. Nature
486 2000;403(6770):665-8. doi: 10.1038/35001088.

487 Pena LA, Fishbein MC. Fatal myocarditis related to Campylobacter jejuni
488 infection: a case report. Cardiovasc Pathol 2007;16(2):119-21. doi:
489 10.1016/j.carpath.2006.09.007.

490 Peters S, Pascoe B, Wu Z, et al. Campylobacter jejuni genotypes are associated
491 with post-infection irritable bowel syndrome in humans. Commun Biol
492 2021;4(1):1015. doi: 10.1038/s42003-021-02554-8.

493 Pönkä A, Pitkänen T, Pettersson T, et al. Carditis and arthritis associated with
494 Campylobacter jejuni infection. Acta Med Scand 1980;208(6):495-6. doi:
495 10.1111/j.0954-6820.1980.tb01237.x.

496 Sher AA, Jerome JP, Bell JA, et al. Experimental Evolution of Campylobacter
497 jejuni Leads to Loss of Motility, rpoN (σ54) Deletion and Genome Reduction.
498 Front Microbiol 2020;11:579989. doi: 10.3389/fmicb.2020.579989.

499 Skogestad J, Aronsen JM. Hypokalemia-Induced Arrhythmias and Heart Failure:
500 New Insights and Implications for Therapy. Front Physiol 2018;9:1500. doi:
501 10.3389/fphys.2018.01500.

502 Spapen J, Hermans H, Rosseel M, et al. Campylobacter jejuni-related
503 cardiomyopathy: Unknown entity or yet underreported? Int J Cardiol
504 2015;198:24-5. doi: 10.1016/j.ijcard.2015.06.110.

505 Turley AJ, Crilley JG, Hall JA. Acute myocarditis secondary to Campylobacter
506 jejuni enterocolitis. Resuscitation 2008;79(1):165-7. doi:
507 10.1016/j.resuscitation.2008.04.021.

508 van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment
509 of Guillain-Barré syndrome. Lancet Neurol 2008;7(10):939-50. doi:
510 10.1016/S1474-4422(08)70215-1.

511 Xu F, Zeng X, Haigh RD, et al. Identification and characterization of a new ferric
512 enterobactin receptor, CfrB, in Campylobacter. J Bacteriol
513 2010;192(17):4425-35. doi: 10.1128/JB.00478-10.

514

Tables

Table 1. *Campylobacter jejuni* virulence genes selected in this study.

Adhesion	*cadF*, *capA*, *flpA*, *ilvE*, *peb1A*, *peb4*, *pgp1*, *porA* and *virB11**
Capsule	*kpsE*, *kpsM* and *pglD*
Colonization	*ggt**
Invasion	*ciaB*, *ciaC*, *ciaD*, *ciaI* and *iamA*
Iron uptake	*ceuE*, *cfrA*, *cfrB** and *fur*
LOS	*cgtB*†, *cst-II**, *cst-III*, *waaF* and *wlaN*
Motility/Chemotaxis	*cheA*, *cheB*, *cheW*, *cheY*, *flaA*, *flaB*, *flaC*, *flgB*, *flhA*, *flhB*, *fliA*, *fliD*, *fliM*, *fliY*, *luxS* and *rpoN*
Response to stress	*ahpC*, *cosR*, *cprR*, *cprS*, *csrA*, *cstA*, *dnaJ*, *hspR*, *htrA*, *htrB*, *katA*, *nuoK*, *perR*, *ppk1*, *ppk2*, *sodB* and *spoT*
T6SS‡	*tssA*, *tssB*, *tssC*, *tssD*, *tssE*, *tssF*, *tssG*, *tssH*, *tssI*, *tssJ*, *tssK*, *tssL* and *tssM*
Toxins	*cdtA*, *cdtB* and *cdtC*

All sequences correspond to reference C. jejuni NCTC 11168 genome, except for:

* *C. jejuni* 81-176 genome,

† *C. jejuni* OH4384 genome and

‡ *C. jejuni* 414 genome.

Note. Adapted from García-Sánchez *et al*, 2019.

525 **Table 2.** Summary of the genome sequence features of the isolated
526 *Campylobacter jejuni* H660 (girl) and H661 (boy) strains and NCTC 11168
527 reference strain.

		H660	H661	NCTC 11168
Assembly	Completeness (%)	99.7	99.7	99.7
	Contigs	24	25	1
	Genome length (bp)	1,667,040	1,666,126	1,641,481
	Genome coverage (X)	800	763	Unknown
	DNA G+C content (%)	30.39	30.39	30.55
MLST	Sequence type	ST-148	ST-148	ST-43
	Clonal complex	CC21	CC21	CC21
Annotation	CDS	1,711	1,708	1,658
	Hypothetical proteins	297	294	221
	rRNA	6	6	9
	tRNA	40	40	44
	CRISPR sequences	0	0	1

528 MLST, multilocus sequence typing. CDS, coding DNA sequence.

529

530 **Table 3.** Phase-variable (PV) tract differences between *Campylobacter jejuni* H660 (girl) and H661 (boy) compared to NCTC 11168

531 genome identified by PhasomeIt v1.1.2 (Aidley *et al*, 2018).

Gene	Gene product	Locus	NCTC11168 PV tract	H660 PV tract	H661 PV tract
kdpB	potassium-transporting ATPase B chain	Chemotaxis	5G+A (ON)	10G (OFF)	9G (ON)
flgR	sigma-54 associated transcriptional activator	Flagella	8A (ON)	7A (OFF)	8A (ON)
wlaN	beta-1,3 galactosyltransferase	LOS	8G (ON)	8G (ON)	9G (OFF)
cj1144c	hypothetical protein Cj1144c	LOS	9A+8G (OFF)	16A+10G (OFF)	12A+9G (OFF)
cj1305c	hypothetical protein Cj1305c (617 family)	Flagella	9G (ON)	9G (ON)	10G (OFF)
cj1310c	hypothetical protein Cj1310c (617 family)	Flagella	9G (ON)	10G (OFF)	9G (ON)
				10G (OFF)	9G (ON)
cj1420c	putative methyltransferase	Capsule	9G (ON)	9G (ON)	10G (OFF)

532 LOS, lipooligosaccharide.