Evaluation of whole-genome sequencing as a genotyping tool for *Campylobacter jejuni* in comparison with pulsed-field gel electrophoresis and *flaA* typing[1]

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ABSTRACT *Campylobacter jejuni* is a leading cause of foodborne illness, with poultry and poultry products being leading sources of infection. Epidemiological efforts to trace *Campylobacter* can be challenging because of the extreme genetic diversity of this bacterium relative to other foodborne pathogens. To enhance tracking and epidemiological efforts, whole-genome sequencing has been used for other foodborne pathogens but not yet been evaluated for practicality with *Campylobacter*. Thus, the purpose of this study was to evaluate whole-genome sequencing as a genotyping method for *C. jejuni* by comparing it with 2 commonly used genotyping methods, namely pulsed-field gel electrophoresis (PFGE) and *flaA* typing. Whole-genome sequence data were generated using the Roche-454 sequencing platform to map *Campylobacter* strains (VOL_3, VOL_5, VOL_8, VOL_11, and VOL_20) isolated from conventional and organic poultry. Five additional isolates with published genomes were also compared. The PFGE profiles were created using Sma I digestion. For the *flaA* short variable region sequencing, standard PCR methods were used and high-quality Sanger reads were generated. The PFGE profiles of strains VOL_3 and VOL_11 were found to be indistinguishable, and strain VOL_20 was found indistinguishable from NCTC 11168. Whole-genome comparisons between strains VOL_20 and 11168 were in agreement with the obtained PFGE profiles, as these 2 isolates had very similar genome sizes, a number of shared genes (1,580), and very similar % G-C content (30.6). Of the 8 strains, 2 strains (VOL_3 and VOL_11) had identical *fla*A types. Whole-genome sequencing was the most discriminatory of the typing methods. However, the cost and time effort needed to sequence and assemble the genomes may hinder efforts, and therefore, we conclude that more bioinformatics tools need to be developed for whole-genome sequencing to be used as an epidemiological tool.

Key words: *Campylobacter*, genome, poultry, pulsed-field gel electrophoresis, *flaA* short variable region

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INTRODUCTION

Campylobacter jejuni is the most significant member of the genus *Campylobacter* because of its pathogenic abilities. The pathogen is gram-negative, thermophilic, spiral-shaped, and fastidious in nutrient and environmental requirements. The bacterium is well-adapted to the environment of the intestinal tract within poultry due to the relatively higher body temperature. The highly studied and sequenced strain 81-176 contains approximately 1,654 genes, about one-half the number of genes present in *Escherichia coli*, and one-third the number of genes present in *Salmonella* Typhimurim (Parkhill et al., 2000). *Campylobacter* is also comparatively very small, about 0.2 to 0.8 microns wide and 0.5 microns long (Debruyne et al., 2008). The reduced genome size is clearly reflected by reduced metabolic abilities because only secondary metabolites, amino acids, or tricarboxylic acid cycle intermediates and not 6-carbon sugars are able to be used. Thus, *Campylobacter* is somewhat dependent on other microorganisms and fares well in microbial communities such as those found in the gastrointestinal tract.

Campylobacter jejuni is a leading cause of human foodborne gastroenteritis and one of the major routes of human campylobacteriosis is assumed to be the con-

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sumption or handling of contaminated poultry meat products (Newell et al., 2001; Scallan et al., 2011). It has been reported that almost 98% of chicken flocks in the United States are colonized by *C. jejuni* (Stern et al., 2001). Levels of colonization have been reported at 10^5 to 10^9 cfu per gram of intestinal contents (Lee and Newell, 2006). Carcass levels of contamination similarly have been reported to vary from 10^2 to 10^5 cfu per carcass (Berndtson et al., 1992). Given these contamination levels and because *C. jejuni* has a low infective dose of about 500 cells, poultry and poultry products possess a high risk of *C. jejuni* infections in humans (Jacobs-Reitsma et al., 2008).

Typing isolates is an important aspect of epidemiological studies for tracing sources and routes of transmission, identifying and monitoring geographical and temporal characteristics, and developing control strategies. Multiple typing methods have been applied including antibiotic resistance profiling, phage typing, serotyping, and several genetically based methods. Although genetic typing methods are becoming more popular, utilization of multiple methods has become necessary due to the enormous genetic variation of *C. jejuni*. With the advances in whole-genome sequencing, a parallel view of multiple genomes is possible. With this approach, information including essential genes for infection and epidemiological tracking data might be mined. The purpose of this study was to evaluate whole-genome sequencing as a genotyping method by comparing it with the 2 most commonly used genotyping methods for *C. jejuni*, pulsed-field gel electrophoresis (**PFGE**) and *flaA* typing.

MATERIALS AND METHODS

Strain Isolation and Characterization

Five strains of *C. jejuni*, 3 isolated from whole raw retail poultry carcasses reared under conventional methods and 2 isolated from whole raw poultry carcasses reared in pastures, were isolated as previously described (Hanning et al., 2010). Briefly, whole carcasses were rinsed in 400 mL of PBS for 2 min using an arcing motion. A 50-mL subsample was centrifuged, and the pellet resuspended in 10 mL of PBS. A 100-μL aliquot of the resuspended pellet was directly plated onto *Campylobacter* Line agar (Line, 2001). The plates were incubated at 42°C under microaerophilic conditions for 48 h. Suspect colonies were confirmed using latex agglutination, via PCR with primers specific for *C. jejuni*, Gram staining, and visual inspection in a microscope under oil immersion objective for spiral shape and typical motility characteristics (Linton et al., 1997). Isolates were also subjected to the hippurate test to differentiate *C. jejuni* from other species of *Campylobacter* (Harvey, 1980). All isolates were passed twice on *Campylobacter* Line agar for purity and stored in 50:50 glycerol prior to genotyping analysis. After isolation, the 3 strains of *C. jejuni* isolated from carcasses reared under conventional methods were assigned the strain identification numbers of VOL_3, VOL_5, and VOL_11, and the 2 strains isolated from carcasses reared under organic pasture flock methods were assigned strain numbers VOL_8 and VOL_20. Five additional strains of *Campylobacter* whose whole genomes have been previously published were included in this study: RM 1221, NCTC 81-176, NCTC 11828 (81116), NCTC 11168, and ATCC BAA-1458 (269.97).

flaA Typing

The *flaA* sequencing of the strains was performed as previously described (Meinersmann et al., 1997). Two sequences were generated for each strain consisting of the entire *flaA* sequence (1,724 bp) and the short variable region (**SVR**; 621 bp). To generate the entire *flaA* sequence, the forward primer FLA4F (5′ GGA TTT CGT ATT AAC ACA AAT GGT GC 3′) and reverse primer FLA1728R (5′ CTG TAG TAA TCT TAA AAC ATT TTG 3′) were used. For the SVR, the primer sets 242F [5′ CTA TGG ATG AGC AAT T(AT)A AAA T 3′] and 625R [5′ CAA G(AT)C CTG TTC C(AT)A CTG AAG 3′] were used. All primers were synthesized by Integrated DNA Technologies (Coralville, IA). For sequencing, PCR amplicons were purified by a QIAquick PCR purification kit (Qiagen, Valencia, CA) and sequenced using the aforementioned primers. Sequencing was carried out in an ABI 3100 capillary analyzing system (Applied Biosystems, Foster City, CA). Sequences were aligned with computer analysis using LaserGene software and the programs EditSeq, SeqMan, and MegAlign software to generate a dendrogram based on sequence similarity by the clustalW method (DNAstar Inc., Madison, WI). The sequences also were compared to a *flaA* database to determine similarities to other strains (http://hercules.medawar.ox.ac.uk/flaA/).

PFGE

The PFGE method was performed according to the CDC PulseNet protocol with modifications specific for *Campylobacter* (Ribot et al., 2001). Briefly, bacteria were cultured on *Campylobacter* agar supplemented with 5% defribinated horse blood. A loop of bacteria was suspended in PBS and optical density adjusted to 0.6 at a wavelength of 630 nm. The suspension was added in a 1:1 ratio to SeaKem gold agarose and proteinase K (100 mg/mL) and dispensed into plug molds (Bio-Rad, Hercules, CA). After solidifying, plug molds were lysed in buffer (50 m*M* Tris, 50 m*M* EDTA (pH 8.0), 1% sarcosine, and 100 mg/mL of proteinase K) at 54°C for 15 min. After cooling, genomic DNA was digested with 40 units of the restriction enzyme SmaI (Clontech, Mountain View, CA.) at 25°C for 2 h according to the manufacturer's instructions. Digested DNA was electrophoresed using a CHEF mapper system (Bio-Rad) with an initial switch time of 6.8 s and final switch time of 38.4 s, voltage of 6 V/cm, and angle

of 120°. Gels were stained with 40 mg of ethidium bromide in a 400 mL solution of 1X TBE for 40 minutes and destained for 10 min in sterile distilled water. Gels were viewed with UV transillumination. Two electrophoresis standards, Low Range PFG and Mid Range I PFG Markers (New England Biolabs, Ipswich, MA) were used to determine and compare the band sizes.

Genome Sequencing

For de novo sequencing, in addition to the 5 strains described in the previous section, the reference strain NCTC 11168 was also sequenced for quality control purposes. Genomic DNA was prepared from isolates cultured as described in the previous section using the Aqua Pure Genomic DNA kit (Bio-Rad). Genome sequencing was performed as previously described by Poly et al. (2007). Briefly, the genomes were sequenced using a 454 Life Sciences GS FLX Titanium series sequencer (Roche Diagnostics, Branford, CT). Strains were sequenced to a depth of 16-fold or greater and were assembled de novo by the 454 Newbler de novo assembler (Roche) to 294 contigs per genome, on average. Statistical analysis using Lander-Waterman methods predicted that more than 99.9% of each genome was sequenced. Sequencing yielded a total of 1,001,614 total reads with a modal read length of 504 bp.

Assembly and Mapping of Genomes

The genomic contigs were ordered and oriented into scaffolds by mapping the contigs against the 2 fully sequenced genomes (NCTC 11168 and RM 1221) using the 454 Newbler software (Roche). A visual inspection of the alignments and mapping was completed for each strain and viewed using EagleView (Huang and Marth, 2008). Gap closures were completed by designing primers (IDT, Coralville, IA) against the ends of the ordered contigs and sequencing PCR products with high-quality Sanger reads (Applied Biosystems, Carlsbad, CA). Regions of repetitive sequence caused the remainder of the assembly gaps as determined by mapping contigs against the fully sequenced strains RM1221 and NCTC 11168.

Genome Annotations and Comparisons

The complete genome sequences for *C. jejuni* strains 81116 (NCTC11828), 269.97, RM 1221, 11168, and 81-176 were accessed under the GenBank accession numbers CP000814, CP000768, CP000025, NC_002163.1, and CP000538, respectively. For all strains in this study, open reading frame (**ORF**) predictions and gene annotation were completed using the Rapid Annotation Using Sub-system Technology (**RAST**) tool version 2.0 (http://rast.nmpdr.org/) automated annotation pipeline (Aziz et al., 2008). Genome comparisons were completed using the SEED viewer version 2.0 for both function-based comparison and sequence-based comparisons (Overbeek et al., 2005). An e-value of 10^{-2} was selected as a threshold for ORF function attribution (Poly et al., 2005). To view genomic rearrangements, sequences were also aligned and viewed using MAUVE version 2.3.1 (Darling et al., 2004).

RESULTS

PFGE

The PFGE patterns produced by the digestion of genomic DNA using the restriction enzyme Sma I were compared with each other and varied from 7 to 10 in total number of bands, and a dendrogram based on the similarity index was produced (Figure 1). The profiles obtained indicated that the number of bands produced did not correlate to the source of the isolate. Two of the newly sequenced isolates (VOL_3 and VOL_11) had indistinguishable PFGE profiles. Strain VOL_20 had a PFGE profile that was indistinguishable from that of *C. jejuni* strain NCTC 11168. The remaining 6 PFGE profiles were all unique.

flaA Typing

The alignment of the *flaA* sequences is shown in Figure 2. We observed that 2 strains VOL_3 and VOL_11 had identical *flaA* SVR sequences and alignment of other 8 strains showed unique patterns dependent on the strain. In this study, we did not observe any relation

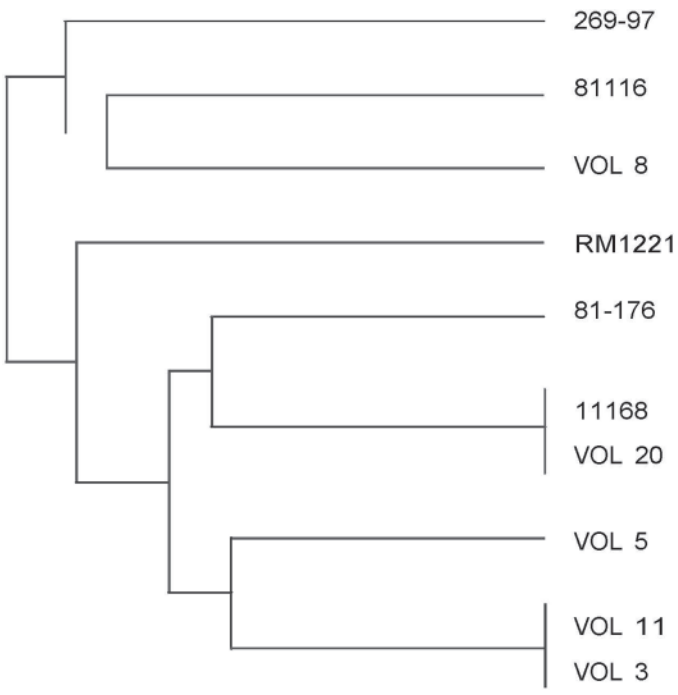

Figure 1. A dendrogram produced by comparing the similarities in pulsed-field gel electrophoretic banding patterns of the 10 strains of *Campylobacter jejuni* used in this study digested with 40 U of the enzyme Sma I.

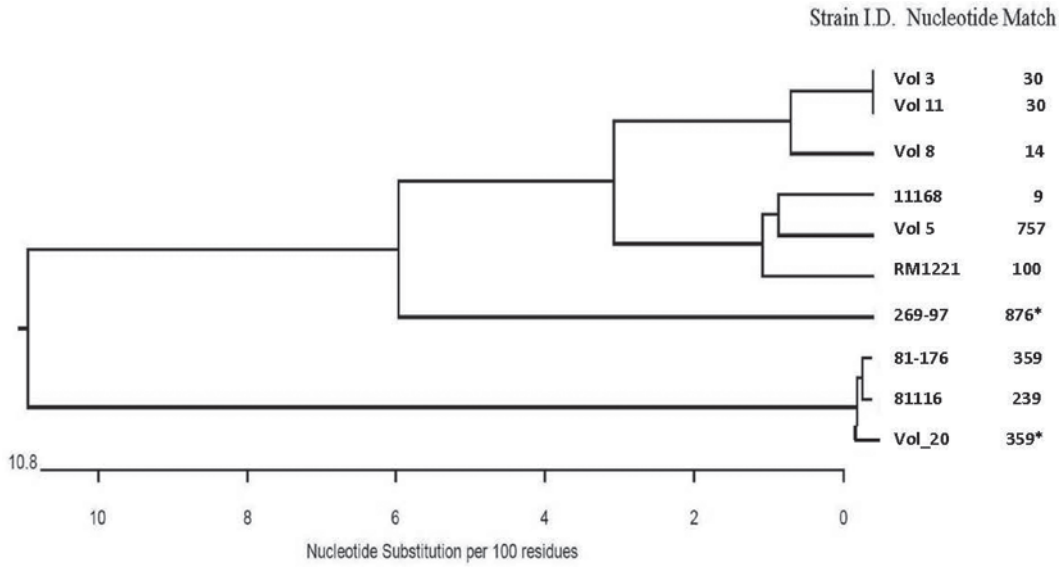

Figure 2. The alignment of the *flaA* short variable region (SVR) of the *Campylobacter jejuni* isolates used and characterized in this study. The asterisks (*) indicate a closest match, whereas all other strains had exact matches for the *flaA* SVR sequence in the multilocus sequencing typing (MLST) database.

with source of isolation and the *flaA* sequencing (similar to the results obtained by PFGE analysis). All the sequences were compared with the *flaA* database found on the MLST organization web page (http://pubmlst.org/campylobacter/). Similarly, from the results of the *fla*A sequencing alignment, 8 of the isolates were given different allele numbers. The strains VOL_3 and VOL_11 were given the same allele number. Strains VOL_20 and 81-176 were given the same allele number; however, VOL_20 was a close match, whereas 81-176 was an exact match to the allele number.

Whole-Genome Sequencing

In this study, we sequenced and mapped 5 strains of *C. jejuni* isolated from retail chicken carcasses and compared these sequences to 5 strains that had sequences previously published. We also searched through the genome and compared the presence or absence of specific genes and subsystems. The purpose was to compare overall similarities in sizes of the genomes in order to determine the core genome and to analyze specific gene and loci sequences for similarities and differences among the strains so that targets might be identified for use in inferring host relationships.

The total genome size of *Campylobacter jejuni* is reported to vary from approximately 1.8 to 1.6 Mb depending on the strain (Parkhill et al., 2000; Miller, 2008). The 5 strains that were sequenced in this study also varied in size but were included in the same range as other sequenced strains (Table 1). The sizes of the genomes were confirmed by the estimates produced by PFGE patterns. All of the poultry isolates had larger total genome sizes than isolates from human clinical illness cases with the exception of strain 269-97, which has the largest genome of all 10 strains compared in this study. The % G-C content of 4 of the strains sequenced in this study were slightly higher (30.8 to 31.2%) than has been reported for the published genomes against which our sequenced genomes were compared (Table 1). However, strain VOL_20 had the same % G-C content as 4 isolates from human illnesses included in this study.

The genomes of the strains that were sequenced in this study were compared to 5 strains with published genomes (81-176, 81116, RM1221, 11168, and 269.97). These published genomes and the newly sequenced strains were also compared with each other with respect to presence or absence of all genes. As expected, genes and subsystems coding for necessary functions (i.e., housekeeping genes) were relatively conserved and present in all genomes compared in this study. Some specific genes present in these strains indicated that these *C. jejuni* isolates had a high degree of genomic plasticity in specific regions. Most of the genes present in the poultry isolates that were absent from the human isolates had unknown or putative functions or

Table 1. Characteristics of the *Campylobacter jejuni* strains sequenced in this study and strains with previously published genomes

Isolate source	Strain	Size (bp)	% G-C
Conventional poultry	VOL_3	1,812,128	31.2
Conventional poultry	VOL_5	1,820,847	31.2
Conventional poultry	VOL_11	1,766,869	31.4
Pasture flock poultry	VOL_8	1,834,433	30.8
Pasture flock poultry	VOL_20	1,669,055	30.6
Poultry isolate	RM1221	1,777,831	30.3
Human	269-97	1,845,106	30.6
Human	81116	1,628,115	30.6
Human	81-176	1,616,554	30.6
Human	11168	1,641,841	30.6

were designated as hypothetical proteins. However, no specific marker genes were identified for host association.

We then calculated the presence or absence of all genes between the genomes similar to other studies (Dorrell et al., 2001) to determine if these comparisons produced useful data for implying host association (Table 2). The number of genes shared between isolates was not a function of isolate source (Table 2). The percentage of genes present in the 5 sequenced isolates that were absent from the other sequenced genes was also calculated (Table 2), which also appeared to be isolate and not source specific. The numbers of genes present in the published sequence strains but absent in the newly sequenced isolates ranged from 62 to 484 (Table 2). Isolate VOL_11 appeared to have the largest number of genes present in the genome that were absent from the other genomes against which it was compared.

It was noticed that isolates VOL_3 and VOL_5 as well as VOL_8 and VOL_20 had similar trends in these presence or absence analyses and that these isolates were obtained from conventionally and organic pasture-raised poultry, respectively. To further investigate this relationship, the isolates were compared and assigned a similarity score of nearest neighbor using RAST (Figure 3). These data were then plotted, and again VOL_3 and VOL_5 as well as VOL_8 and VOL_20 tended to cluster, whereas VOL_11 appeared to be unique. This same trend was apparent regarding % G-C content but not genome size.

DISCUSSION

Most recently, whole-genome sequencing was applied to the *E. coli* O104:H4 strain that caused the world's largest foodborne illness outbreak due to *E. coli* (CDC, 2011; Life Technologies Corporation, 2011). Whole-genome sequencing was used to identify and design a strain-specific assay. Furthermore, the data obtained from sequencing allowed identification of specific virulence genes. With this approach, the data were available within a week and this method expedited tracking and detection efforts.

We evaluated this same approach as an epidemiological tool for *C. jejuni*. For this study, we compared our newly sequenced strains with 4 isolates from human infection (81-176, 81116, NCTC 11168, and 269-97) and 1 strain isolated from poultry (RM 1221). We included the sequenced strain 269-97 *C. jejuni* subspecies *doylei* and 4 other strains that are subspecies *jejuni*. Subspecies *doylei* has been isolated from blood, more often than subspecies *jejuni*; thus, possible information about virulence potential might be obtained by comparing this strain with others (Parker et al., 2007).

We used PFGE and *flaA* typing as genotyping methods against which to compare whole-genome sequencing. We found that PFGE did not differentiate between strain VOL_20 and NCTC strain 11168. However, *flaA* SVR sequencing did produce unique sequences for these 2 strains. Similarly, Laturnus et al. (2005) analyzed 6 *flaA* types, but found only 3 restriction pattern types of the isolates studied in their work. For genotyping,

Table 2. Comparisons of genes in *Campylobacter jejuni* isolates sequenced in this study and published genomes of *C. jejuni*: number of shared genes, percentage of unique genes present in newly sequenced strains, and number of genes absent from newly sequenced strains

Isolate	VOL_3	VOL_5	VOL_8	VOL_11	VOL_20	Average
No. of shared genes between *C. jejuni* isolates						
RM1221	1,592	1,568	1,725	1,489	1,569	1,588.6
11168	1,528	1,509	1,587	1,394	1,580	1,519.6
81-176	1,511	1,493	1,587	1,369	1,558	1,503.6
81116	1,505	1,485	1,570	1,369	1,529	1,491.6
269-97	1,596	1,676	1,641	1,680	1,684	1,655.4
VOL_3	X	1,789	1,687	1,638	1,544	1,664.5
VOL_5	1,789	X	1,659	1,721	1,662	1,680.6
VOL_8	1,687	1,659	X	1,575	1,669	1,634.3
VOL_11	1,638	1,721	1,575	X	1,516	1,604.0
VOL_20	1,544	1,662	1,669	1,516	X	1,615.6
Unique genes in the newly sequenced *C. jejuni* isolates (%)						
RM1221	18	20	18	22	11	19.6
11168	19	20	18	21	13	19.7
81-176	17	19	16	15	12	16.3
81116	14	16	9	22	11	14.8
269-97	16	18	16	22	14	17.7
VOL_3	X	7	15	19	14	13.75
VOL_5	7	X	16	17	14	13.5
VOL_8	15	16	X	22	7	15
VOL_11	19	17	22	X	21	19.75
VOL_20	14	14	7	21	X	14
No. of genes present in previously published sequenced strains but absent from the newly sequenced strains						
RM1221	245	269	112	348	268	
11168	156	185	62	308	56	
81-176	142	160	66	284	95	
81116	129	150	64	265	101	
269-97	374	398	308	484	415	

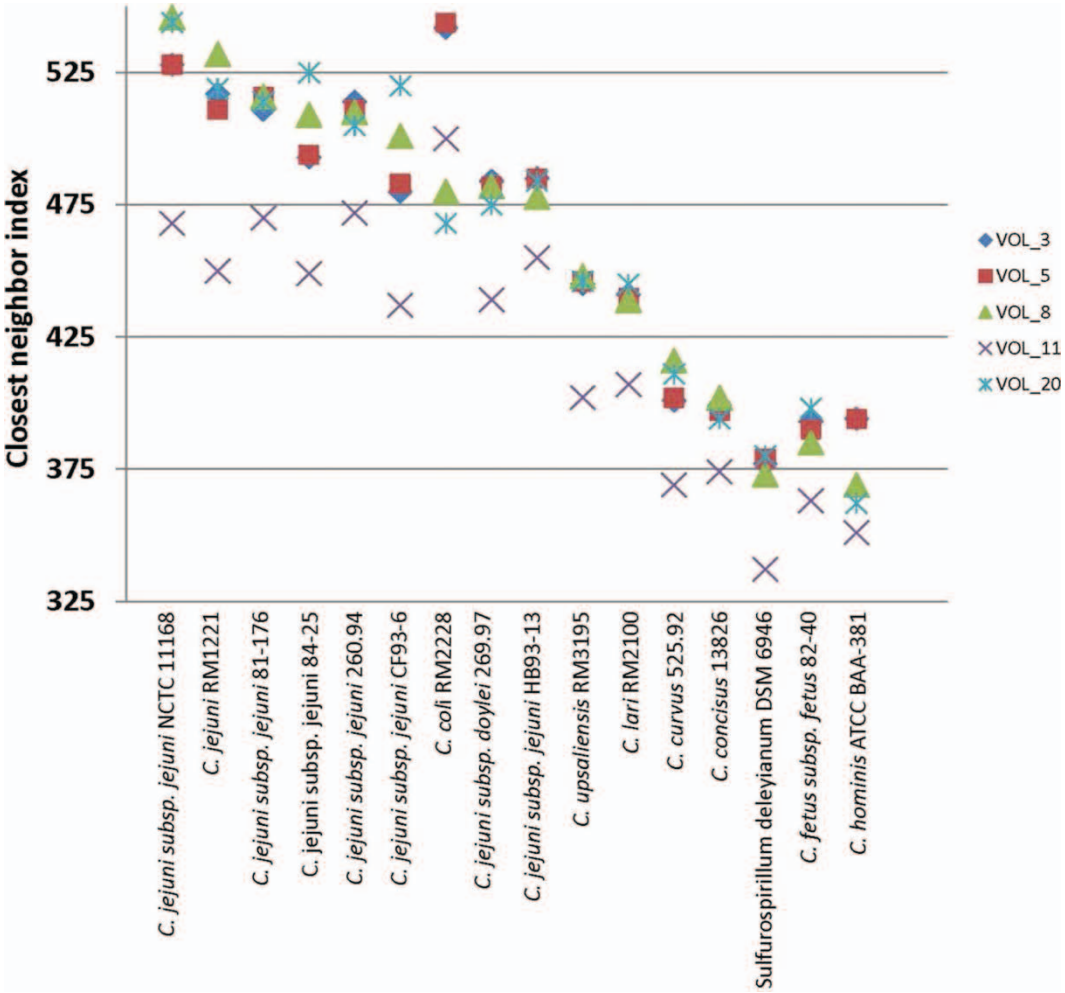

Figure 3. A scatter plot of the nearest neighbor similarity index among all the strains of *Campylobacter*. Color version available in the online PDF.

PFGE is the gold standard and considered to be more reliable than *flaA* sequencing (Huang et al., 2005). Not surprisingly, whole-genome sequencing data indicated that all 10 strains used in this study were unique strains and thus whole-genome sequencing was the most discriminatory. Overall, the clonal relationship of the isolates appeared to be different and dependent on the method used.

As presented in the Results section, we were not able to categorize any of the isolates using a specific target. This is similar to other reports of comparisons between poultry and human isolates of *C. jejuni* (Poly et al., 2005, 2007; Parker et al., 2006). One problem with finding a marker that might be suitable for associating the bacteria with a host is that many of the genes present in *C. jejuni* isolated from poultry are absent from the *C. jejuni* isolated from human infections. This finding supported the previously published reports (Dorrell et al., 2001; Parker et al., 2006). Dorrell et al. (2001) used DNA microarray hybridization techniques and determined roughly 21% of the genes present in poultry strains were absent from the human strains used in that study. Similarly, Parker et al. (2006) used comparative genomic hybridization and identified 4 large *Campylobacter jejuni*-integrated elements that were absent from the NCTC 11168 human strain. Using a shotgun DNA microarray, Poly et al. (2005) found unique genes in all 3 isolates that they compared.

The numbers and percentages of genes present or absent were analyzed and shown in Table 2. Similar data have been presented in genome comparison studies of *Campylobacter* as a useful measure of comparison that may reflect similarities and imply more similar genomes with smaller percent absence or larger numbers of shared genes (Dorrell et al., 2001). However, the results of our analysis indicate that these measurements are biased and are a reflection of a comparison between large to small genomes. Thus, the actual core-genome (1,456 genes) shared among all strains may be a more accurate number to use when comparing all the genomes. This number of core genes is similar to that reported by Poly et al. (2007), who compared 3 genomes and found that the core number of genes among their isolates was 1474.

One noticeable difference between isolates from poultry and humans was the differences in % G-C content. With the exception of VOL_20, the 4 poultry isolates

sequenced in this work had % G-C contents ranging from 30.8 to 31.4%, which was higher than the G-C content of the human isolates. The higher % G-C content of these poultry isolates may be due to larger genomes than the human isolates and many of these genes may have been acquired by mechanisms including horizontal gene transfer (Chan et al., 2008). This was apparent when examining the genome for insertion sequences because phage coding sequences including a putative phage lysozyme and phage integrase were present in 4 of the newly sequenced strains but not VOL_20.

Our study and others have found differences in G-C content in *C. jejuni* genomes of approximately the same size (Champion et al., 2008). It could be implied that isolates with lower G-C content may have some type of functional differences compared with those isolates with higher G-C content (Gu et al., 1998). Because *Campylobacter* is a thermophilic bacterium, a high G-C content of thermostable amino acids may play an adaptive role to help inhabit relatively hot niches (Argos et al., 1979; Kagawa et al., 1984). Indeed, the body temperature of the chicken is 42°C, whereas human body temperature is 37°C. But, it is not clear from this study if this advantage is truly related to an adaptive advantage for host inhabitation, and thus, further research will be needed to understand this finding.

Genomic rearrangement has been suggested as a method of survival against stress for C. *jejuni* (Newell et al., 2001). The presence of multiple copies of interspacer sequences observed in our isolates suggests that a high frequency of recombination may be possible within the *Campylobacter* genome (Hanning et al., 2010). Most recently, Stahl and Stintzi (2011) used transposon methodology and found that essential genes of *Campylobacter* were not located in the same positions along the chromosome in different strains. This confirmed the genomic plasticity in our findings as well as previous reports. This particular point makes whole-genome sequencing advantageous over some other genotyping methodologies (*flaA* sequencing, MLST) because genomic rearrangements could not be identified by flaA sequencing or MLST and thus differing strains might be misidentified as clones.

For *C. jejuni*, the surprising degree of intrastrain diversity revealed through genome sequencing shows that each genome contains a sample of the genes potentially available to members of a given population. It appears that the pan-genome of *C. jejuni* has yet to be completely identified primarily due to the incredible amount of genomic diversity (Lefébure et al., 2010). Given that *C. jejuni* isolated from different animal taxa (cattle, swine, and wild birds) appear to have unique genotypes, it will be interesting to see if isolates from these animals have any new genes that might contribute to the pan-genome as a whole (Gonzalez et al., 2009). Thus, whole-genome sequencing approaches offer opportunities to collect data beyond a genotyping method.

Two potential factors that need improvement were noticed during the course of this research. First, many of the proteins in the *C. jejuni* genome have unknown, hypothetical, or putative functions (Stahl and Stintzi, 2011). Much information is lacking about the mechanisms of how *C. jejuni* causes disease and colonize poultry. Perhaps investigations and elucidation of these proteins with unknown functions will further the knowledge about this pathogen. Second, although the sequencing technology is becoming less expensive, the main cost associated whole-genome sequencing lies in the man hours needed to assemble the genome. Thus, improved tools are needed in order to expedite the assembly of genomes. The availability of more analytical tools may facilitate the use of whole-genome sequencing as an epidemiological tool.

In conclusion, we found that the relationship among the strains was specific to 1 of the 3 typing methods used and none of the typing methods completely agreed on the clonal relationship among the strains. Whole-genome sequencing provided unique genotyping data including genome size, percent G-C content, and identification of genomic rearrangements. Because of the genomic variability and plasticity of *C. jejuni*, genotyping methods such as MLST and *flaA* typing would not be able to detect gene rearrangements and could falsely identify different strains as clones. However, both MLST and *flaA* have advantages over other genotyping methods and whole-genome sequencing that include a relatively lower cost and time required to use these typing methods. The major limitation of whole-genome sequencing at the present time is the cost in terms of man hours spent preparing, sequencing, and analyzing samples. Thus, for whole-genome sequencing to be feasible as a typing tool, new and specific tools need to be developed.

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