Comparison of Molecular Subtyping and Antimicrobial Resistance Detection Methods Used in a Large Multistate Outbreak of Extensively Drug-Resistant *Campylobacter jejuni* Infections Linked to Pet Store Puppies

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ABSTRACT *Campylobacter jejuni* is a leading cause of enteric bacterial illness in the United States. Traditional molecular subtyping methods, such as pulsed-field gel electrophoresis (PFGE) and 7-gene multilocus sequence typing (MLST), provided limited resolution to adequately identify *C. jejuni* outbreaks and separate out sporadic isolates during outbreak investigations. Whole-genome sequencing (WGS) has emerged as a powerful tool for *C. jejuni* outbreak detection. In this investigation, 45 human and 11 puppy isolates obtained during a 2016–2018 outbreak linked to pet store puppies were sequenced. Core genome multilocus sequence typing (cgMLST) and high-quality single nucleotide polymorphism (hqSNP) analysis of the sequence data separated the isolates into the same two clades containing minor within-clade differences; however, cgMLST analysis does not require selection of an appropriate reference genome, making the method preferable to hqSNP analysis for *Campylobacter* surveillance and cluster detection. The isolates were classified as sequence type 2109 (ST2109)—a rarely seen MLST sequence type. PFGE was performed on 38 human and 10 puppy isolates; PFGE patterns did not reliably predict clustering by cgMLST analysis. Genetic detection of antimicrobial resistance determinants predicted that all outbreak-associated isolates would be resistant to six drug classes. Traditional antimicrobial susceptibility testing (AST) confirmed a high correlation between genotypic and phenotypic antimicrobial resistance determinations. WGS analysis linked *C. jejuni* isolates in humans and pet store puppies even when canine exposure information was unknown, aiding the epidemiological investigation during the outbreak. WGS data were also used to quickly identify the highly drug-resistant profile of these outbreak-associated *C. jejuni* isolates.

KEYWORDS *Campylobacter*, antibiotic resistance, canine, core genome, molecular epidemiology, multilocus sequence typing, subtyping, whole-genome sequencing

Campylobacter is a leading cause of bacterial foodborne and zoonotic illness in the United States, resulting in an estimated 1.5 million illnesses, 19,300 hospitalizations, and 240 deaths annually (S. A. Collier, L. Deng, E. A. Adam, K. M. Benedict, A. Blackstock, B. B. Bruce, G. Derado, W. C. Edens, K. E. Fullerton, J. W. Gargano, A. Geissler, A. J. Hall,

V. R. Hill, R. M. Hoekstra, N. P. Nelson, S. Reddy, E. Scallan, E. K. Stokes, J. S. Yoder, and M. J. Beach, submitted for publication). *Campylobacter jejuni* is the most common *Campylobacter* species associated with human illness (1, 2). Though *Campylobacter* cases are usually sporadic, previous *Campylobacter* infection outbreaks have been linked to consumption of raw milk, contaminated water, chicken meat, and raw peas (3–7). Exposure to pets such as dogs also carries a risk of *Campylobacter* infection (1–3, 8, 9). During 2012 to 2017, 13 canine-associated *Campylobacter* infection outbreaks were reported through the National Outbreak Reporting System (NORS) (https://wwwn .cdc.gov/norsdashboard/) to the Animal Contact Outbreak Surveillance System. Studies have shown *C. jejuni* may cause diarrhea in dogs, especially puppies, and that dogs can carry *C. jejuni* asymptomatically (9).

Pulsed-field gel electrophoresis (PFGE) and 7-gene multilocus sequence typing (MLST) have been used historically to differentiate sporadic from outbreak *C. jejuni* isolates (4, 5, 10, 11). However, whole-genome sequencing (WGS) has been shown to provide superior resolution and concordance with epidemiologic data compared with PFGE and MLST during these outbreak investigations (12–16). WGS provides information across a broader range of the genome and is therefore more informative than methods such as PFGE and MLST, which provide information from limited parts of the genome (17, 18). WGS analysis methods used in *Campylobacter* outbreak investigations can include (i) high-quality single nucleotide polymorphism (hqSNP) analysis, which compares isolate genomes to a closely related reference to derive SNP differences; (ii) core genome MLST (cgMLST) analysis, which examines allele differences at core genome loci of the isolates (loci that are found in 95% of the reference organism strains); and (iii) whole-genome MLST (wgMLST) analysis, which examines allele differences between core and accessory genome loci of the isolates (13, 19–21). Since cgMLST examines allele differences in genes that are common to all isolates being compared, it should be well suited to surveillance. If further resolution between potential outbreak isolates is needed, then hqSNP or wgMLST can be used (22, 23).

Most patients recover from *Campylobacter* infections without antibiotic treatment; however, antibiotic treatment is recommended in patients with severe illness or risk factors for severe disease, such as age over 65 years, pregnancy, or immune suppression (24). Broth microdilution is the preferred method used for antimicrobial susceptibility testing (AST) of *Campylobacter* isolates (25). The method is not performed by many laboratories and is time-consuming for *Campylobacter* isolates, which can take 48 h to grow and require a microaerophilic environment (26). WGS analysis is a timely, cost-effective method to identify resistance determinants and accurately predict the corresponding phenotypes (23, 27–29). Recent work with *Campylobacter* has demonstrated a strong correlation between resistance determined by AST and resistance predicted through the identification of known genetic resistance determinants from WGS data (25).

In this study, we examined sequences from 56 outbreak-associated *C. jejuni* isolates obtained from 45 human and 11 puppy fecal specimens during a multistate outbreak linked to pet store puppies (30). We compared the abilities of PFGE, 7-gene MLST, cgMLST, and hqSNP analyses to identify outbreak isolates after the conclusion of the investigation. We identified resistance determinants using WGS data and inferred a predicted resistance pattern, which was compared with phenotypic AST results for a subset of isolates.

MATERIALS AND METHODS

Isolates. From January 2016 through February 2018, *C. jejuni* isolates from 51 stool specimens and 23 puppy fecal specimens were obtained; however, only 45 human and 11 puppy isolates were consistent with the case definition described by Montgomery et al. (30). Human stool specimens or isolates were submitted to public health laboratories (PHLs) from physician offices or clinical laboratories according to state-specific requirements. In addition, puppy fecal specimens were collected by PHLs and agriculture officials from 29 pet stores in six states. Isolate information is listed in Table S1 in the supplemental material.

Pulsed-field gel electrophoresis. PFGE was performed by PHLs or at CDC for a subset of isolates obtained from human and puppy fecal specimens using the standard operating procedure for PulseNet

PFGE of *Campylobacter jejuni* (17). The DNA was digested with restriction enzyme SmaI (48 isolates), and in some cases DNA was also digested with restriction enzyme KpnI (35 isolates). PFGE patterns were analyzed in BioNumerics v6.6.10 (Applied Maths, Sint-Martens-Latem, Belgium) and uploaded to the PulseNet *Campylobacter* National Database.

Whole-genome sequencing and analysis. Genomic DNA was extracted from overnight cultures of the isolates listed in Table S1, and the DNA was sequenced by PHLs or CDC according to the standard operating procedure for PulseNet Nextera XT Library Prep and Run setup for the Illumina Miseq (https://www.cdc.gov/pulsenet/pdf/pnl32-miseq-nextera-xt.pdf). The sequences were analyzed in Bio-Numerics v7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium), and cgMLST was performed on assemblies that were 1.6 to 1.8 Mbp in length, had ≥49× base pair coverage, and had 88 to 98% of core genome (1168 to 1316) loci identified that were within PulseNet quality control thresholds (1.4 to 2.2 Mbp long, ≥20× base pair coverage, and ≥85% of core genome loci identified). The cgMLST scheme (available at http://pubmlst.org/campylobacter) in the BioNumerics database contains 1,343 *C. jejuni*/*C. coli* loci (20). Cluster analysis using cgMLST allele calls from loci identified in all 56 sequences (categorical values) was used to generate an unweighted pair group method using average linkages (UPGMA) dendrogram. Loci without allele calls were ignored in pairwise sequence comparisons used to generate the tree.

To generate a closed reference sequence, genomic DNA from a representative isolate included in the largest cgMLST clade (2017D-0132) was also extracted from an overnight culture using a Promega (Madison, WI) Wizard genomic-DNA purification kit. Library size selection was completed using Bluepip-pen (Sage Science, Beverly, MA). The library was sequenced on a Pacific Biosciences (PacBio) (Menlo Park, CA) RSII instrument using one SMRT cell and P6-C4 chemistry for 360 min. The sequence was initially assembled using the PacBio hierarchical genome assembly process version 3 (31). The mean coverage of the PacBio sequence was 488.3×, and the chromosomal contig (1.67-Mbp sequence length; 30.6% guanine-cytosine content [GC]) and two plasmid contigs (contig 1, 55.9-kbp sequence length and 28.4% GC; contig 2, 31.9-kbp sequence length and 28.3% GC) were circular and complete. Unicycler-polish v0.4.4 with Pilon v1.22 (https://github.com/rrwick/Unicycler) was used to polish this assembly using the Illumina sequence reads for the isolate (32, 33).

HqSNP analysis was performed by trimming the Illumina sequence reads, using fastx_trimmer, three bases from the 5′ ends and mapping the sequences to the assembled chromosomal PacBio sequence using Lyve-SET version 1.1.4f with SMALT (34, 35). SNPs were called using VarScan (36) at >20× coverage and >95% read support and allowed flanking set to 100 bp (21). To provide a side-by-side visual comparison of the cgMLST and hqSNP sequence analyses, a tanglegram was generated in R v3.5.1 (https://github.com/rstudio/rstudio) using the dendextend package (37). Several layout optimization methods were evaluated (DendSer, labels, ladderize, random, step1side, and step2side), and among these, the step2side method produced the best result and was used for optimization (https://github .com/jchen232/CampyPuppyTangle). *In silico* 7-gene MLST analysis of sequences was performed using a feature in BioNumerics v7.6, which provides the MLST STs from the *Campylobacter* MLST database (http://pubmlst.org/campylobacter).

Acquired and mutational antimicrobial resistance determinants in the isolates were detected by analyzing the assembled sequences and through read-based approaches, as previously described (38). Briefly, resistance genes were detected using Megablast in NCBI-blast+ v. 2.3.0 and the ResFinder database (updated on 2 March 2018) with cutoffs of 90% identity and 50% coverage (39). Read mapping to 23S rRNA and *gyrA* reference sequences (NCBI accession numbers Z29326.1 and NC_002163.1 [positions 957631 to 960222], respectively) in CLC Genomics Workbench v11.0 (Qiagen, Redwood City, CA) was used to detect mutations in the genes. Predicted phenotypes were assigned based on the presence or absence of resistance determinants. Lastly, predicted resistance heat maps based on the cgMLST UPGMA tree constructed in BioNumerics v7.6 were generated in R v3.5.1 using the gheatmap function in the ggtree package (40).

Antimicrobial susceptibility testing. The MICs of nine antimicrobials (azithromycin, ciprofloxa-cin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline) belonging to 7 antimicrobial classes on the National Antimicrobial Resistance Monitoring System (NARMS) *Campylobacter* panel were determined for 19 human and 9 puppy isolates (see Fig. 2) using a standard broth microdilution assay (Sensititre; Thermo Fisher Scientific, Waltham, MA), and the results were interpreted using clinical breakpoints or epidemiological cutoff values (ECOFF) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/ clinical_breakpoints).

Resistance classification. Isolates are reported as "susceptible" and "resistant" here according to the EUCAST criteria (http://www.eucast.org/, accessed 15 February 2020). Briefly, isolates were categorized as susceptible if they had either an MIC in the susceptible range according to clinical breakpoints or a wild-type MIC according to the ECOFF. Isolates were categorized as resistant if they had either a resistant result according to clinical breakpoints or an MIC above the ECOFF (not wild type). Although there is not currently an accepted definition of what constitutes extensive drug resistance in *C. jejuni*, there is growing awareness of the need to distinguish strains with resistance to multiple antimicrobials (increasingly common in the United States) from those for which treatment options are limited to broad-spectrum antibiotics rarely required for the management of *Campylobacter* infections (41). Here, we use the term "extensively drug resistant" (XDR) to refer to strains that are resistant to macrolides and fluoroquinolones (the antimicrobial classes recommended for treatment of *Campylobacter*) and three or more additional antimicrobial classes (42).
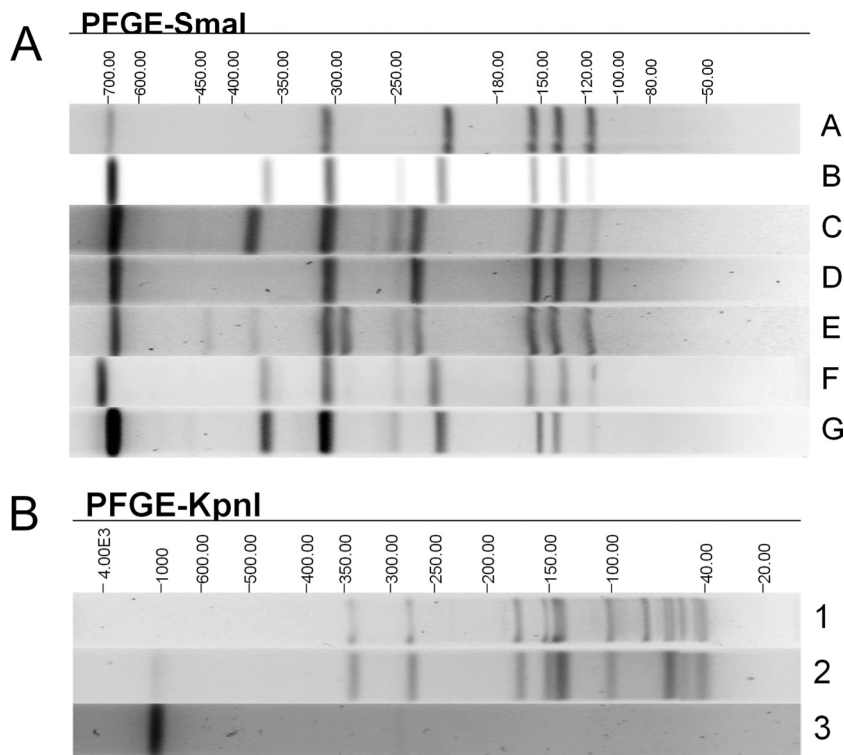
FIG 1 Representative SmaI (A) and KpnI (B) PFGE patterns of the *C. jejuni* isolates associated with the outbreak. Different PFGE patterns are designated A to G (SmaI) or 1 to 3 (KpnI). Molecular sizes (in kilobases) are displayed above the gels.

Accession number(s). Illumina sequence reads were deposited in the Sequence Read Archive at NCBI with the accession numbers shown in Table S1. The closed reference genome of *C. jejuni* isolate 2017D-0132 (Biosample no. SAMN07615386) was deposited in GenBank at NCBI with the following accession numbers: chromosomal contig, CP047082; plasmid contig 1, CP047083; and plasmid contig 2, CP047084).

RESULTS

PFGE analysis. SmaI PFGE performed on a subset of 38 human and 10 puppy isolates resulted in seven different patterns, designated A to G (Fig. 1 and 2). KpnI PFGE performed on 25 human and 10 puppy isolates resulted in three different patterns, designated 1 to 3, and 10 different SmaI/KpnI pattern combinations (Fig. 1 and 2). Isolates not analyzed with KpnI were provided a KpnI pattern designation of 0, and isolates not analyzed by PFGE were designated NP, for PFGE not performed (Fig. 2).

MLST, cgMLST, and hqSNP analyses. Using *in silico* MLST, we determined that all of the sequenced isolates (45 human and 11 puppy isolates) were sequence type 2109 (ST2109). These isolates formed two distinct clades (clades 1 and 2) by cgMLST analysis; each clade contained isolates from cases both with and without known exposures to pet stores and puppies, with clade allelic differences ranging from 0 to 40 (Fig. 2). Clades 1 and 2 were differentiated from each other by 201 to 241 alleles. Several isolates that were separated by two-enzyme PFGE clustered together by cgMLST analysis. Isolates with pattern combinations A2 and B1 fell into cgMLST clade 1 only; isolates producing pattern combinations C3 and D3 fell into cgMLST clade 2 only; and only one isolate each produced pattern combinations B3, E3, F3, and G3 (located in clade 2 only). In addition, isolates in cgMLST clade 1 had four different PFGE pattern combinations (A1, A2, A3, and B1), and isolates in cgMLST clade 2 had seven different PFGE pattern combinations (A3, B3, C3, D3, E3, F3, and G3). Conversely, several isolates that were indistinguishable by SmaI/KpnI PFGE pattern combinations were dispersed into different clades by cgMLST. Isolates with pattern combination A3 were found in clades 1 and 2. The sequences were also examined using hqSNP analysis. A tanglegram
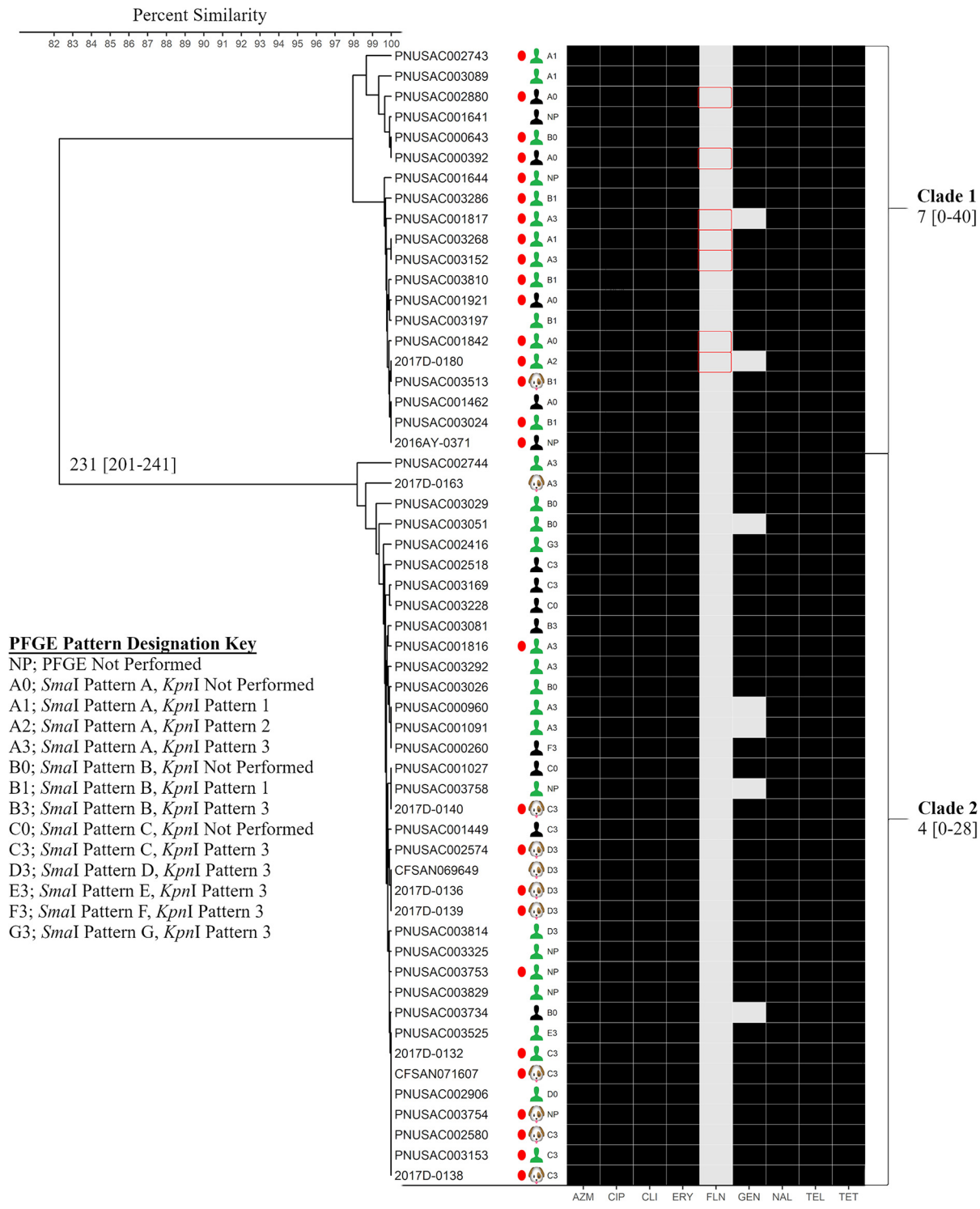
FIG 2 CgMLST and antimicrobial resistance analyses of *C. jejuni* isolates. Shown is a UPGMA dendrogram containing sequences from 45 human and 11 puppy isolates constructed from cgMLST loci (1,316 loci) in BioNumerics v7.6.3. SmaI and/or KpnI pattern designations of isolates from Fig. 1 are shown in the PFGE column, and a PFGE pattern designation key is provided. Median (minimum-maximum) allele differences among sequences within clades 1 and 2 and between clades are shown. Percent similarities of allele calls from the sequences are represented by the scale at the upper left. Isolates from human cases with an epidemiologic link to puppies from pet stores (green torsos), human cases without any epidemiologic information available (black torsos), and puppy feces (puppy heads) are indicated. Predicted antimicrobial resistance derived from *in silico* resistance determinant detection is indicated by the shaded boxes: azithromycin (AZM), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), florfenicol (FLN), gentamicin (GEN), nalidixic acid (NAL), telithromycin (TEL), and tetracycline (TET). Black shading indicates that the isolate was predicted to be resistant to the antimicrobial drug, and light-gray shading indicates that the isolate was predicted to be susceptible to the antimicrobial drug. Traditional antimicrobial susceptibility testing was also performed on the isolates indicated by red circles. Red outlining of boxes indicates that the isolates were predicted to be susceptible to the antimicrobial drug but that they were determined to be resistant to the antimicrobial drug by traditional AST.
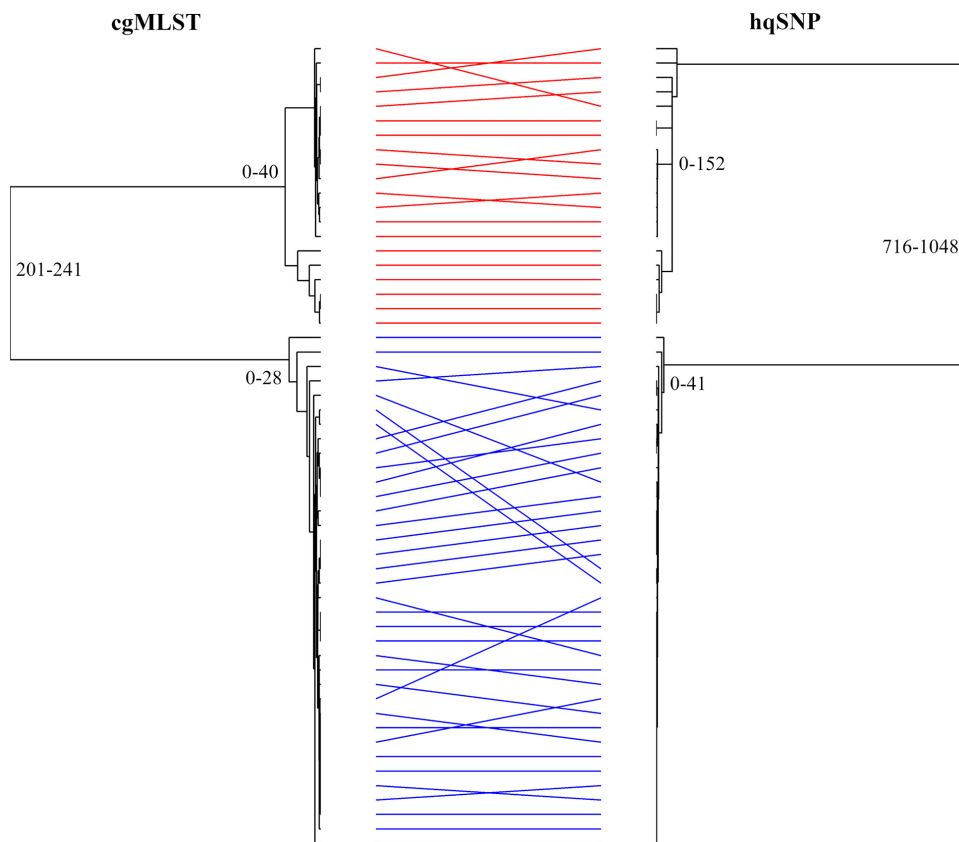
FIG 3 Side-by-side comparison of cgMLST and hqSNP dendrograms generated from the sequences of the *C. jejuni* outbreak isolates. The tanglegram was created with R v3.5.1 using the dendextend package, and the layout was optimized using the step2side method. Identical strains are linked between the trees using straight lines that are colored according to the cgMLST clade: red, clade 1; blue, clade 2. Minimum-maximum allele or SNP differences among sequences within clades 1 and 2 and between clades are shown.

demonstrated that the isolates formed the same two clades, as observed by cgMLST analysis (0 to 152 and 0 to 41 SNPs), and hqSNP analysis differentiated clades 1 and 2 by 716 to 1,048 SNPs; however, minor differences in within-clade placement of isolates were observed (Fig. 3).

Antimicrobial resistance. All 56 outbreak isolates contained genes or mutations encoding resistance to kanamycin/amikacin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid, erythromycin, azithromycin, telithromycin, and clindamycin. Furthermore, 49 isolates contained a gene encoding resistance to gentamicin (Table 1). The 28 isolates that were further characterized by AST using the NARMS *Campylobacter* panel

TABLE 1 Genotypic and phenotypic antibiotic resistance of *C. jejuni* isolates from humans and puppies

Drug class	Drug	Presence of gene(s) or mutation(s) conferring resistance (n = 56)	No. of isolates phenotypically resistant (n = 28)	Correlation between genotype and phenotype (%)
Tetracyclines	Tetracycline	*tet* (O) (n = 56)	28	28/28 (100)
Aminoglycosides	Kanamycin/amikacin	*aph* (3')-III (n = 56)	NT[a]	NT[a]
Aminoglycosides	Streptomycin	*aad* E (n = 56)	NT[a]	NT[a]
Aminoglycosides	Gentamicin	*aph* (2″)-Ih (n = 49)	26	28/28 (100)
Phenicol	Florfenicol	None	7	21/28 (75)
Quinolones	Ciprofloxacin	*gyr* A T86I (n = 56)	28	28/28 (100)
	Nalidixic acid	*gyr* A T86I (n = 56)	28	28/28 (100)
Macrolides	Azithromycin	23S rRNA A2075G (n = 56)	28	28/28 (100)
	Erythromycin	23S rRNA A2075G (n = 56)	28	28/28 (100)
Ketolides	Telithromycin	23S rRNA A2075G (n = 56)	28	28/28 (100)
Lincosamide	Clindamycin	23S rRNA A2075G (n = 56)	28	28/28 (100)

[a]NT, not tested; kanamycin/amikacin and streptomycin are not tested on the NARMS AST panel.

showed resistance to tetracycline, ciprofloxacin, nalidixic acid, erythromycin, azithromycin, telithromycin, and clindamycin, and 26/28 isolates had resistance to gentamicin (Table 1). The correlations between the resistance phenotypes and genotypes for the tested drugs were 100%, except for florfenicol (Table 1). Seven of 28 isolates were phenotypically resistant to florfenicol; however, no genetic determinants associated with florfenicol resistance were found in these isolates (Table 1 and Fig. 2). Kanamycin, amikacin, and streptomycin are not on the NARMS *Campylobacter* panel, so the correlation between resistance phenotypes and genotypes for these drugs could not be determined (Table 1).

DISCUSSION

WGS data analysis methods have previously been shown to provide greater concordance with epidemiologic data during outbreak investigations and demonstrate higher throughput, reproducibility, and sensitivity than results generated by 7-gene MLST and PFGE (12–16, 43, 44). In this investigation, we showed that cgMLST analysis of WGS data was highly concordant with epidemiologic information linking cases to a *Campylobacter* outbreak associated with puppies. Additionally, cgMLST identified some cases genetically related to the outbreak strain where epidemiologic linkage could not be determined or cases were lost to follow-up. Two main clades of isolates were identified using cgMLST analysis, whereas 7-gene MLST grouped all the isolates into the same ST. Specifically, all the isolates in this outbreak investigation were ST2109, which is a rare sequence type in the ST45 clonal complex (45–48). However, eight additional ST2109 isolates (from three chicken samples and five clinical specimens) were not genetically related to the isolates in cgMLST clades 1 and 2 (>50 alleles different), had unknown epidemiologic linkage to the outbreak isolates, and did not produce the XDR resistance patterns necessary for outbreak inclusion (data not shown), suggesting that 7-gene MLST alone is not discriminatory enough to be used during *Campylobacter* outbreak investigations. PFGE analysis, another traditional subtyping technique, resulted in an array of diverse patterns among the epidemiologically linked cases and thus could not provide the strong concordance that was provided by WGS for confirmed cases in this puppy-linked *Campylobacter* outbreak. The diverse PFGE patterns may be due to most isolates exhibiting partial digestion using SmaI and no digestion using KpnI, suggesting the presence of methylases, which affects the interpretation of the PFGE data. In addition, point mutations, recombinations, insertions, and deletions can lead to the loss or gain of restriction sites, causing related isolates to have distinct PFGE banding patterns (49). Therefore, it is not surprising that sequences generated from isolates with diverse PFGE patterns clustered together by cgMLST analysis.

The *C. jejuni* isolates identified over a 2-year period fell into two distinct cgMLST clades with high taxon diversity within and between clades. Animal contact *Campylobacter* outbreaks have been shown to contain isolates with a large amount of genetic diversity, suggesting that host-adapted evolution of strains may occur or that multiple strains of the same pathogen may infect a host animal (4, 5, 11). Conversely, *Campylobacter* outbreaks associated with raw milk have been shown to contain clinical isolates with limited genetic diversity (6, 7, 16, 50). As sequence-based analysis methods such as cgMLST become more widely used to investigate *Campylobacter* outbreaks, a better understanding of the potential genetic diversity of isolates will develop.

There were some differences in the ordering of the isolates within the individual clades in the cgMLST tree compared with the hqSNP tree, which was expected, as cgMLST and hqSNP methods measure relatedness of isolates differently (i.e., alleles versus SNPs); however, the isolates grouped the same within each clade. The ranges of SNP differences among isolates within each clade and between clades were greater than the range of allele differences identified using cgMLST. This may be due to several reasons, including SNPs in the intergenic region, multiple SNPs within a single gene, and SNPs detected in genomic regions outside the cgMLST scheme. HqSNP analysis is dependent on *a priori* knowledge of isolates to select an appropriate reference genome, whereas only the genus and species of the isolates are needed before they can

be analyzed by cgMLST against an organism-specific allele database, making cgMLST preferable for surveillance and cluster detection of *Campylobacter*.

The *C. jejuni* isolates included in this outbreak investigation were predicted to be resistant to 10 or 11 drugs across six drug classes, including azithromycin and ciprofloxacin, the antimicrobials recommended by the Infectious Diseases Society of America for the treatment of *Campylobacter*-infected patients (42). These resistance patterns had been rarely seen among *C. jejuni* isolates previously; of more than 12,000 *C. jejuni* surveillance isolates submitted to NARMS from 2004 to 2015, only 1% had resistance patterns similar to those observed among isolates in this outbreak (https://wwwn.cdc .gov/narmsnow/). Interestingly, many puppies associated with the outbreak were administered prophylactic antibiotics to which the strains were resistant, including macrolides, tetracyclines, quinolones, and aminoglycosides (30); this practice may have provided a selective advantage for the XDR *C. jejuni* strains.

As mentioned above, all of the *C. jejuni* outbreak isolates were ST2109 and had similar XDR patterns, implying that the XDR profile is a distinguishing feature of this ST. However, a recent study found that an ST2109 isolate from a dog was resistant to azithromycin, clindamycin, erythromycin, gentamicin, telithromycin, and tetracycline but susceptible to quinolones and florfenicol (45). In addition, the eight ST2109 isolates unrelated to the outbreak were not XDR (data not shown), suggesting that identification of ST2109 alone would not be sufficient to predict the XDR resistance pattern of *Campylobacter* isolates. Selective pressure from antibiotic use in puppies where ST2109 is found should be explored as a possible explanation for the emergence of XDR ST2109.

Predicted resistances using WGS and antimicrobial susceptibility testing were concordant for all drugs except florfenicol for which both WGS and MIC data were available. None of the 28 isolates examined contained a known florfenicol resistance determinant, but 7 isolates were phenotypically florfenicol resistant, suggesting a possible unknown resistance mechanism, which is being further investigated. The correlation between predicted resistance and AST results in this study is similar to results from a 2016 study where correlation between WGS-derived antimicrobial genotypes and corresponding AST phenotypes were examined among 114 *Campylobacter* isolates (25). Specifically, there was 100% agreement for tetracycline, ciprofloxacin, nalidixic acid, and erythromycin and 95 to 99% agreement for gentamicin, azithromycin, clindamycin, and telithromycin resistance. However, all the isolates in that study were also florfenicol susceptible by AST, and no genes for florfenicol resistance were found (25).

In conclusion, we demonstrated that we were better able to link *C. jejuni* isolates from humans and pet store puppies using WGS-based analysis methods than using traditional subtyping methods. WGS data were used to quickly identify XDR profiles and resistance determinants in a broader set of isolates than those that would be tested phenotypically. These results demonstrate the power of WGS to aid in *C. jejuni* outbreak investigations and predict the antimicrobial resistance phenotype of the isolates.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

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