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Utility of Multilocus Sequence Typing as an Epidemiological Tool for Investigation of Outbreaks of Gastroenteritis Caused by *Campylobacter jejuni*

Andrew D. Sails,* Bala Swaminathan, and Patricia I. Fields

Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

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Multilocus sequence typing (MLST) has been proven useful for the study of the global population structure of *Campylobacter jejuni*; however, its usefulness for the investigation of outbreaks of disease caused by *C. jejuni* has not been proven. In this study, MLST plus sequencing of the *flaA* short variable region (SVR) were applied to 47 isolates from 12 outbreaks of *C. jejuni* infection whose relatedness has been determined previously, and the results were compared to those of serotyping and pulsed-field gel electrophoresis (PFGE). Isolates implicated in an outbreak were indistinguishable by all four subtyping methods, with sporadic isolates being distinguished from outbreak isolates. Two sporadic isolates from one outbreak were resistant to *Sma*I digestion and therefore nontypeable by PFGE but were differentiated from the outbreak strain by the other methods. PFGE and *flaA* SVR typing were the most discriminatory methods, with discriminatory indices (DI) of 0.930 and 0.923, respectively. However, an epidemic strain from one outbreak was distinguished from the other outbreak isolates by *flaA* SVR typing; its *flaA* allele was different at five nucleotides, suggesting that this change was possibly mediated by recombination. MLST was less discriminatory than PFGE and *flaA* SVR typing (DI = 0.859), and many of the epidemic strains possessed common sequence types (STs) including ST-8, -21, -22, and -42. However, further discrimination within STs was achieved by *flaA* SVR typing or PFGE. The results from this study demonstrate that a combined approach of MLST plus *flaA* SVR typing provides a level of discrimination equivalent to PFGE for outbreak investigations.

Campylobacter jejuni is a pathogen that primarily causes gastrointestinal disease in humans. Gastrointestinal infection with *C. jejuni* causes significant morbidity and mortality, with the estimated number of cases a year in the United States exceeding 2 million and an estimated 2,000 deaths attributed to the infection (21). Human gastrointestinal infection by *C. jejuni* is thought to occur through the zoonotic transmission of the organism from natural reservoirs via food or water. *C. jejuni* forms part of the normal gastrointestinal flora of many farm animals, including poultry, pigs, and cattle (2), and is also found in environmental surface waters (3). Most human infections are sporadic (31); however, outbreaks do occur occasionally.

Contaminated milk has been responsible for large outbreaks of *C. jejuni* enteritis (24). Contamination of milk can be caused by direct excretion from an asymptomatic cow with mastitis (17) or, more commonly, by fecal contamination during milking (43). The majority of outbreaks have occurred after the consumption of raw milk (44), although improperly pasteurized milk (1) and postpasteurization contamination have been implicated as sources of outbreaks. Consumption of untreated surface water and cross-contamination from raw or undercooked products to other foods have also been demonstrated to be responsible for outbreaks of infection (34).

The detection of outbreaks of gastrointestinal disease caused by *C. jejuni* is important for the identification of the source or vehicle of infection and for limiting further infection in the community. Accurate and sensitive subtyping methods are required if we are to recognize outbreaks of infection, to match case isolates with those from potential vehicles of infection, and to discriminate these from unrelated strains.

A multitude of subtyping methods have been described for *C. jejuni*. Phenotypic methods include serotyping (32), phage typing (36), and biotyping (4). A number of molecular subtyping methods have been developed for *C. jejuni*, including ribotyping (10), *flaA* restriction fragment length polymorphism typing (25), amplified fragment length polymorphism typing (5), random amplified polymorphic DNA (19), and pulsed-field gel electrophoresis (PFGE) (28). PFGE has proven to be useful and discriminatory for the investigation of outbreaks of gastroenteritis caused by *C. jejuni* (11) and is considered to be the current "gold standard." Ribot and colleagues (33) described a rapid standardized 1-day protocol for PFGE typing of *C. jejuni* that may facilitate timely investigation of outbreaks. Standardized protocols for PFGE and computer-assisted comparison of the PFGE patterns facilitates interlaboratory comparisons and has enabled the development of a nationwide surveillance network (PulseNet) within the United States, which has also aided in the detection of outbreaks of food-borne disease (39). However, interlaboratory comparisons of PFGE patterns require strict adherence to complex, standardized protocols, thereby limiting these comparisons to laboratories that have access to the appropriate equipment and software.

DNA sequencing-based methods for subtyping virtually eliminate experimental variation, facilitating interlaboratory comparisons. The text data generated by these methods can be

* Corresponding author. Present address: Health Protection Agency, Newcastle Laboratory, Institute of Pathology, Newcastle General Hospital, Westgate Rd., Newcastle Upon Tyne, NE4 6BE, United Kingdom. Phone: 44 191 226 1074. Fax: 44 191 226 0365. E-mail: andrew.sails@hpa.org.uk.

TABLE 1. Demographic information for the 12 outbreaks included in this study

Outbreak no.	Location	Date (yr)	Source	No. of isolates	Comment(s)	Reference
1	Kansas	1998	Food handler	6	161 cases at a school luncheon	27
2	Florida	1983	City water supply	5	865 cases	35
3	Kansas	1981	Raw milk (dairy A)	6		18
4	Vermont	1983	Raw milk	4		15
5	Vermont	1982	Raw milk	2	15 cases	40
6	Vermont	1986	Inadequately pasteurized milk	4	104 cases at a boarding school	1
7	Oklahoma	1988	Fecal contamination of milk	5	104 cases in 4 prisons	Not previously reported
8	New York	1986	Tuna salad	4	Prison outbreak	Not previously reported
9	Kansas	1988	Raw milk	4	87 cases at a bible school	Not previously reported
10	Wisconsin	1995	Tuna salad	2	79 cases at a summer camp	34
11	Maine	1989	Food handler	3	Restaurant associated	Not previously reported
12	Louisiana	1995	Contaminated garlic bread	2	30 cases, restaurant associated	Not previously reported

easily transmitted electronically via the internet and compared to DNA sequences of other strains, allowing the development of global databases. The development of automated DNA sequencers and significant reductions in the price of reagents has led to an increasing use of DNA sequence data for routine clinical applications. DNA sequencing-based subtyping methods have been reported for *C. jejuni*. Sequencing a 267-bp segment of the flagellin (*flaA*) gene of *C. jejuni*, including the short variable region (SVR) may be useful for the investigation of *C. jejuni* outbreaks (22). Dingle and colleagues sequenced a 321-bp segment of the *flaA* gene that included the SVR in a study investigating the genetic diversity of *C. jejuni* strains associated with Guillain-Barré syndrome (7). This study identified 20 different SVR nucleotide sequences in 25 strains, illustrating the variability present in the sequence.

Multilocus sequence typing (MLST) is a subtyping method that is analogous to multilocus enzyme electrophoresis. Similarly to multilocus enzyme electrophoresis, MLST indexes the neutral genetic variation in housekeeping genes, which evolve slowly and are not under selective pressure. This technique was originally described for *Neisseria meningitidis* (20) but has been applied to other organisms, including *C. jejuni* (6). The sequences of 400- to 500-bp segments of seven housekeeping genes are determined by using automated sequencing, and the alleles from the seven genes are assigned a number based upon those already described in the *Campylobacter* database accessible through the internet at www.mlst.net. The 7-digit number, or allele profile, is assigned a sequence type (ST) based on those already present in the database, with novel allele profiles being assigned new STs arbitrarily.

MLST has been useful for investigating the DNA sequence diversity within *C. jejuni* and estimating the rates of intraspecies recombination (6, 38). MLST has also been proven very useful for longitudinal studies of population structure. However, the utility of MLST as an epidemiological tool for the investigation of outbreaks of disease caused by *C. jejuni* has not been established. The sequencing of more variable genes in conjunction with MLST has proven useful in investigations of outbreaks of *N. meningitidis* infection and may increase the discriminatory power of the method (9). The aim of the present study was to compare the subtyping of 47 isolates from 12 *C. jejuni* outbreaks by using PFGE, serotyping, MLST, sequencing of the 321-bp region of the *flaA* SVR, and a combined approach of MLST and SVR sequencing in an attempt to increase the discriminatory power of the DNA sequencing-based method.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. *C. jejuni* isolates were obtained from the National *Campylobacter* and *Helicobacter* Reference Laboratory, Centers for Disease Control and Prevention. Forty-seven isolates were included in the study from 12 separate outbreaks (Table 1). Prior to testing, we recovered *C. jejuni* isolates from storage at −70°C and cultured the isolates on heart infusion agar containing 5% (vol/vol) defibrinated rabbit blood (Becton Dickinson Biosciences, Sparks, Md.). We incubated all cultures under microaerobic conditions at 37°C for 48 h.

HS serotyping. The isolates had been previously serotyped at the National *Campylobacter* and *Helicobacter* Reference Laboratory, Centers for Disease Control and Prevention, by using the heat-stable (HS) serotyping scheme of Penner and Hennessy. The serotyping results of seven of the outbreaks were previously reported (1, 11, 29, 32).

PFGE. We carried out PFGE typing by using *Sma*I to macrorestrict DNA and electrophoretic conditions as previously described by Ribot and colleagues (33). Electrophoresis was carried out by using a CHEF mapper (Bio-Rad Laboratories, Hercules, Calif.). Following electrophoresis, we visualized the fragments by ethidium bromide staining and UV transillumination. We captured gel images with a Gel Doc 2000 image analysis system (Bio-Rad Laboratories). PFGE gel images were normalized, and PFGE profiles were grouped together by using the Dice coefficient and clustering with the unweighted pair group method with arithmetic averages by using BioNumerics (version 3.0; Applied Maths, Austin, Tex.). Isolates were assigned to the same PFGE macrorestriction profile (mrp) when they clustered at greater than 95% similarity (0.41% optimization and 1.5% position tolerance), and we considered isolates differing at one band as different, with types being numbered arbitrarily (e.g., mrp 1 and 2, etc.). We confirmed the results of the clustering visually.

***flaA* SVR and MLST sequencing.** We carried out MLST as previously described by Dingle and colleagues (6). Briefly, we suspended a 1-μl loop of cells from a fresh overnight plate culture of the isolate in 0.5 ml of reagent grade water. We then heated the cell mixture in a heating block at 100°C for 15 min to lyse the cells. The mixture was centrifuged at 19,000 × *g* for 10 min, and we carefully removed 10 μl of the supernatant and added it to 90 μl of reagent grade water in a 0.5-ml microtube. We used PCR to amplify fragments of the seven housekeeping gene loci by using the reaction conditions and primers of Dingle and colleagues (6) and then amplified the entire *flaA* gene with consensus primers (42). The presence of the correct size PCR product was confirmed by gel electrophoresis. The PCR products were purified with a QiaAmp PCR purification kit according to the manufacturer's instructions (Qiagen, Valencia, Calif.). The purified products were diluted (1:9, vol/vol) in reagent grade water. Sequencing reaction mixtures were prepared with 3 μl of BigDye reaction mix (Applied Biosystems, Foster City, Calif.), 11 μl of the diluted PCR product, and 1 μl of primer to give a final concentration of 3.2 pmol per reaction mixture. Sequencing of the 7 MLST loci (*asp*A, *gln*A, *glt*A, *gly*A, *pgm*, *tkt*, and *unc*A) was carried out by using the previously described primers (6), and the *flaA* SVR was sequenced by using the following novel primers: *flaA* 130, GAT GCT TCA GGG ATG GCG ATA; *flaA* 624, CAA GTC CTG TTC CAA C(TA)G A(AG)G; *flaA* 612, GTT CCA ACT GAA GTT GAA ATC ACA. The reaction products were

TABLE 2. Comparison of MLST, PFGE, and *flaA* SVR sequencing for the discrimination of outbreak-associated *C. jejuni* isolates

Isolate	HS type(s)	PFGE mrp	ST	*flaA* SVR type	Outbreak no.	Source	EI[a]
D5482	19	1	22	6	1	Human	Y
D5479	19	1	22	6	1	Human	Y
D5475	19	1	22	6	1	Human	Y
D5476	19	1	22	6	1	Human	Y
D5480	19	1	22	6	1	Human	Y
D5478	4	2	48	13	1	Human	N
D0445	19	3	22	6	2	Human	Y
D0450	19	3	22	6	2	Human	Y
D0452	19	3	22	6	2	Human	Y
D0462	19, 23, 34	NT[b]	470	15	2	Wild bird	N
D0467	19, 23, 34	NT	470	15	2	Wild bird	N
EDL18	36, 23, 22	4	42	9	3	Human	Y
EDL22	36, 23, 22	4	42	9	3	Human	Y
SSU9892	36, 23, 22	4	42	9	3	Cow, dairy A	Y
SSU9894	36, 23, 22	4	42	9	3	Cow, dairy A	Y
SSU9896	2	5	21	3	3	Cow, dairy A	N
EDL2	36, 23	4	42	9	3	Cow, dairy B	N
D1117	2	6	21	4	4	Child	Y
D1118	2	6	21	4	4	Milk from cow A	Y
D1114	2	6	21	4	4	Calf (drank cow A's milk)	Y
D1108	4	7	38	7	4	Feces from cow A	Y
D0224	2	6	21	4	5	Human	Y
D0226	2	6	21	4	5	Cow feces	Y
D2286	2	8	21	3	6	Human	Y
D2287	2	8	21	3	6	Human	Y
D2290	2	8	21	3	6	Cow feces	Y
D2289	36, 23, 22	9	42	11	6	Cow feces	N
D2643	1, 8	10	8	1	7	Human	Y
D2642	1, 8	10	8	1	7	Human	Y
D2651	1, 8	10	8	1	7	Human	Y
D2641	1, 8	10	8	1	7	Cow feces (feed barn II)	Y
D2640	23, 36	4	42	10	7	Cow feces (feed barn I)	N
D2248	55	11	45	12	8	Human	Y
D2256	55	11	45	12	8	Human	Y
D2253	4, 13	12	122	14	8	Human	N
D2261	2	8	21	3	8	Human	N
D2669	2	8	21	3	9	Human	Y
D2678	2	8	21	3	9	Human	Y
D2692	2	8	21	5	9	Milk sample	Y
D2677	15, 23, 36	13	459	11	9	Cow feces	N
D5157	33	11	679	12	10	Human	Y
D5161	33	11	679	12	10	Human	Y
D2763	2	14	8	2	11	Human	Y
D2770	2	14	8	2	11	Human	Y
D2769	2	14	8	2	11	Human	Y
D5163	41	15	41	8	12	Human	Y
D5166	41	15	41	8	12	Human	Y

[a] EI, epidemiological implication; Y, yes; N, no.
[b] NT, not typeable, did not restrict with *Sma*I.

purified by column purification (Centri Sep 96; Princeton Separations, Adelphia, N.J.). We then separated and detected these reaction products with an ABI Prism 377 automated DNA sequencer (Applied Biosystems). Chromatograms were exported into SeqMan II (DNAStar Inc., Madison, Wis.), and the forward and reverse sequences were assembled. We cut the sequences to previously described allele lengths (between 402 and 507 bp) for each of the 7 MLST loci and then assigned allele numbers based on those already described in the MLST database at www.MLST.net. STs were assigned based upon those already present in the MLST database. The *flaA* SVR sequences were cut to 321 nucleotides, corresponding to nucleotides 283 to 603 of the coding sequence as previously described (7) and assigned allele numbers or *flaA* SVR types arbitrarily in order of description based on their nucleotide sequences.

DI. Simpson's index of diversity (DI) was calculated for each of the subtyping methods by using the formula of Hunter and Gaston (16).

Nucleotide sequence accession numbers. We submitted the partial *flaA* sequences to GenBank (accession numbers AY226587 to AY226590). Sequences that were identical to other sequences in GenBank were not submitted to the database.

RESULTS

Strain discrimination within outbreaks. A summary of the results of the subtyping methods is presented in Table 2. In general, isolates within an outbreak were indistinguishable from each other by all four subtyping methods. Further, geographically and temporally related isolates that had been shown not to be part of the outbreak were differentiated from outbreak isolates, with the following exceptions. (i) Two isolates from wild birds associated with the second outbreak were resistant to *Sma*I digestion and therefore nontypeable by PFGE, but they were differentiated from the outbreak strains by MLST, serotyping, and *flaA* SVR typing. (ii) One isolate (Table 2, EDL2) that was not epidemiologically implicated in outbreak 3 was indistinguishable from the outbreak strains by

TABLE 3. DIs for *C. jejuni* subtyping methods

Method	No. of types	No. of isolates with the most common type/total no. of isolates (%)	DI
Serotyping	12	16/47 (34)	0.839
PFGE	15	7/47 (15)	0.930
MLST	12	13/47 (28)	0.859
flaA SVR	15	8/47 (17)	0.923
MLST and *flaA* SVR	17	8/47 (17)	0.924

all methods except for the detection of an additional HS antigen, HS:22. This antigen can be variably detected, and the sporadic strain was considered the same serotype as the outbreak strains (29). (iii) In outbreak 4, a fecal isolate from a cow implicated in the raw milk outbreak was found to be distinct from the outbreak strain by all four methods; however, a milk isolate from the same cow matched the outbreak strains. (iv) In outbreak 9, an epidemiologically implicated milk isolate was differentiated from other outbreak isolates by *flaA* SVR typing but was found to be indistinguishable by other typing methods. The *flaA* allele from the milk isolate contained five single-nucleotide polymorphisms (SNPs) relative to the outbreak strain in a 150-nucleotide-long segment located at the 3′ end of the 329 bases sequenced.

Strain discrimination between outbreaks by each method. The DI of the subtyping methods varied between 0.839 for serotyping and 0.930 for PFGE (Table 3). We identified 12 HS serotypes among the 47 isolates. The most common serotype was HS:2, which was the serotype of the epidemic strains in outbreaks 4, 5, 6, 9, and 11 and was also the serotype of temporally related strains from two other outbreaks. Serotype HS:19 was the second most common serotype and was the serotype of the epidemic strains in outbreaks 1 and 2. Serotype HS:36,23,22 was the serotype of the epidemic strain in outbreak 3.

PFGE identified 15 mrp's with *Sma*I, with some outbreak patterns associated with more than one outbreak; the number of bands per mrp varied between four and nine (Fig. 1). The most common type was mrp 8, which was the type of the epidemic strains in outbreaks 6 and 9 and a temporally related strain in outbreak 8. The epidemic strain in outbreak 3 was indistinguishable from a temporally related strain from outbreak 7 (mrp 4). The epidemic strains in outbreaks 4 and 5 (mrp 6) and 8 and 10 (mrp 11) were indistinguishable.

MLST discriminated the isolates into 12 STs, with some STs associated with more than one outbreak. The epidemic strains from outbreaks 1 and 2 were both ST-22. The epidemic strain from outbreak 3, plus temporally related strains from outbreaks 3, 6, and 7 were all ST-42. The epidemic strain from outbreaks 4, 5, 6, and 9 plus temporally related strains from outbreaks 3 and 8 were all ST-21. The epidemic strain from outbreaks 7 and 11 was ST-8. The DI was 0.859 for MLST; however, if the *flaA* SVR results were included as an additional allele, the DI was increased to 0.924.

We discriminated the isolates into 15 subtypes by using *flaA* SVR sequencing. Some subtypes were unique to each outbreak set; however, some were common to more than one outbreak: SVR type 6 was the subtype identified in the epidemic strains of outbreaks 1 and 2; SVR type 4 was the epidemic subtype in the strains from outbreaks 4 and 5; SVR type 3 was the subtype of the outbreak 6 epidemic strains and also a temporally related strain from outbreak 3; SVR type 12 was identified in the epidemic strains of outbreaks 8 and 10.

Discrimination within ST by PFGE and *flaA* SVR sequencing. Some of the isolates indistinguishable by MLST were further discriminated by using PFGE or *flaA* SVR sequencing (Table 4). Four STs, ST-8, -21, -22, and -42, were separated into two or more PFGE types and into between two and four types by *flaA* SVR sequencing. The differences between the SVR sequences within each of the STs varied by between 1 and 24 SNPs.

DISCUSSION

Serotyping with the Penner HS scheme has been successfully used to investigate outbreaks of *C. jejuni* disease for many years (29); however, this method has a number of limitations that restrict its use mainly to reference laboratories. Serotyping requires the production of a large panel of antisera, which is labor intensive and expensive. Furthermore, this method has relatively poor discriminatory power and suffers from problems with cross-reactivity between antigens. Approximately 20% of strains are nontypeable by this method (42). The strains in this study were previously serotyped by using the Penner serotyping scheme, and none were found to be nontypeable. The serotypes of the strains in this study included HS:1, HS:2, HS:4, HS:19, and HS:55, all common serotypes frequently found among *C. jejuni* strains from sporadic infections in the United States (30). Serotyping correctly distinguished sporadic isolates from outbreak isolates in 11 of the 12 outbreaks; it failed to differentiate the sporadic strain EDL2 in outbreak 3 from the epidemic strain, as did the other three subtyping methods. In this study, Penner serotyping was the least discriminatory subtyping method (DI = 0.839) for strain discrimination among all of the 47 strains from the 12 outbreaks. Previous studies have found that using Penner serotyping in conjunction with another subtyping method such as phagetyping or Lior (heat-labile) serotyping can increase the discriminatory power of subtyping (36, 45).

In this study, PFGE correctly differentiated temporally re-

TABLE 4. Discrimination within STs by PFGE and *flaA* SVR sequencing

ST	PFGE mrp	*flaA* SVR type	No. of isolates	No. of nucleotide differences (SNPs) between SVR types
ST-8	10	1	4	11
	14	2	3	
ST-21	5	3	1	4, 5, or 7
	6	4	5	
	8	3	6	
	8	5	1	
ST-22	1	6	5	NA[a]
	3	6	3	
ST-42	2	9	1	1, 11, 23, or 24
	4	9	4	
	4	10	1	
	9	11	1	

[a] NA, not applicable.

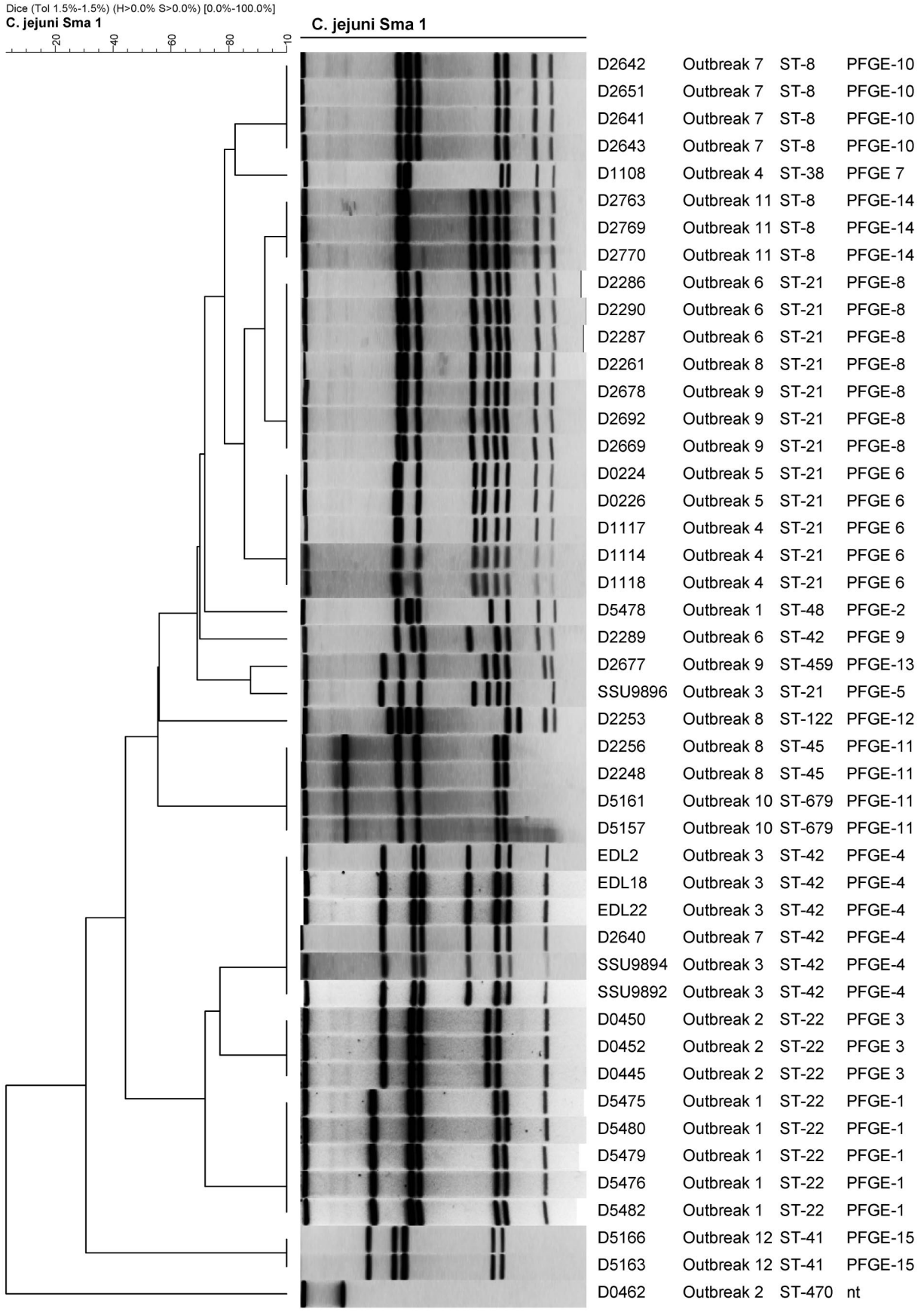

FIG. 1. *C. jejuni* outbreak isolates clustered by using the unweighted pair group method with arithmetic averages of PFGE profiles. The PFGE profile from strain D0467 was not included due to lack of restriction by *Sma*I (nt).

lated isolates from outbreak strains in 10 of the 12 outbreaks. It did not distinguish isolate EDL2 in outbreak 3, as described above. DNA from two bird isolates in outbreak 2 did not restrict with the *Sma*I enzyme, and therefore, these strains could not be typed by PFGE. The inability of restriction enzymes to cut DNA from some isolates may reduce the sensitivity of PFGE among some strains (26). PFGE was the most discriminatory subtyping method in this study (DI = 0.930); however, some strains from separate outbreaks had indistinguishable PFGE profiles. The PFGE profiles reported in this study with *Sma*I contained between 4 and 9 bands per profile. PFGE subtyping of *C. jejuni* by using *Sma*I with profiles based upon <10 bands may limit the discriminatory power of the method. Further studies of the prevalence and diversity of *C. jejuni Sma*I PFGE types of isolates from sporadic human infections in the United States are required to validate the usefulness of this method. In a recent study, the restriction enzyme *Kpn*I was found to be more discriminatory than *Sma*I (23). In addition, the *Kpn*I clustering of strains related to ingestion of raw milk and specific water sources correlated better, leading the authors to conclude *Kpn*I to be the enzyme of choice for molecular epidemiological studies of *C. jejuni* (23).

In our study, we used a rapid PFGE protocol that can be completed in 24 to 30 h, (33) unlike previous methods that require 4 to 5 days to complete the analysis. However, even rapid protocols are labor intensive, and PFGE requires considerable financial investment, both in analysis equipment to generate the profiles and in computer software to normalize the data for interlaboratory comparisons. PFGE profiles may not provide a stable fingerprint; chromosomal rearrangements may lead to minor or major changes in profiles, making this method unsuitable for long-term or global epidemiological studies of *C. jejuni* (12, 41).

Sequencing the SVR of the *flaA* gene of *C. jejuni* has been useful both for investigating outbreaks of human disease (22) and for investigating the genetic diversity of *C. jejuni* strains within poultry operations (14). The variation in *flaA* indexed by SVR typing is thought be under diversifying selection, thereby making it a suitable target for short-term epidemic investigations (8). However, studies have demonstrated that *flaA* can undergo intra- and intergenomic recombination (13); thus, the instability in this locus may make *flaA* SVR typing unsuitable either for epidemic or for longitudinal epidemiological studies. In this study, *flaA* SVR typing correctly differentiated sporadic isolates from outbreak isolates in 10 of the 12 outbreaks. In outbreak 9, one of the outbreak strains was distinguished from the others by *flaA* SVR typing. The isolate had several nucleotide substitutions distributed throughout a 150-nucleotide region of the SVR, suggesting that the change was due to recombination rather than spontaneous mutation. Thus, this outbreak may be an example of why *flaA* typing alone may not be adequate for epidemiologic investigation of outbreaks.

MLST indexes the variation in housekeeping genes, which accumulates slowly since the genes are under stabilizing selection to conserve metabolic function. This method has been found to be very useful for investigating the genetic diversity of *C. jejuni* and for estimating the role of recombination in shaping its population structure (6, 8, 38); however, its utility for short-term epidemiological or outbreak investigations has not been established. In a recent study, 12 isolates of *C. jejuni* representing three outbreaks were typed using amplified fragment length polymorphism, MLST, and clustered regularly interspersed short palindromic repeat typing (37). All three methods were reported to be equally useful for identifying strains from outbreaks; however, the epidemic strains from each outbreak possessed very common STs (ST-21, -45, and -53), suggesting that recognition of outbreaks caused by strains with common STs may be problematic. In this study, MLST was as discriminatory as PFGE for distinguishing temporally related isolates from the epidemic strain in all of the outbreaks. However, MLST was not as discriminatory as PFGE for strain discrimination between outbreaks (DI = 0.859). Incorporating the *flaA* SVR sequencing results as an additional locus provided increased discrimination within the more common STs and raised the DI to 0.923, a DI very similar to that provided by PFGE.

Theoretically, MLST and other DNA sequencing-based approaches have the potential for high intra- and interlaboratory reproducibility. MLST, with its standardized nomenclature, the unambiguous nature of sequence data, and the potential for long-term global comparisons via a centralized database represents a significant advantage over PFGE and other gel-based methods. However, the method in its current form is rather unwieldy and labor intensive, requiring the sequencing of >3,000 bp per isolate. Generation of an ST requires the combination of 14 separate sequencing reactions or experiments; therefore, sequencing errors may lead to the generation of incorrect data. A single nucleotide sequencing error can lead to the designation of a new allele and ST; therefore, great care must be exercised when setting up reactions and loading samples on sequencing gels. Sequencing the allele in both directions can reduce the likelihood of such errors, and strict controls must be maintained to allow potential new alleles to be deposited in global databases only after the original sequencing results are reconfirmed and the original sequencing traces are sent to the database curator.

The results from this study demonstrate that a combined approach of MLST plus sequencing of the *flaA* SVR provides a level of discrimination equivalent to that of PFGE for outbreak investigations. Determining the ST of strains will also facilitate global comparisons via the centralized database, providing long-term epidemiological data for future comparisons. Although PFGE may be less laborious and costly when compared to MLST, reductions in cost and improvements in sequencing technology, including further automation of the technique, will make MLST more accessible for routine clinical microbiology laboratories in the future. Incorporation of additional variable loci such as the *flaA* SVR will provide a level of discrimination suitable for outbreak investigations while providing data suitable for longitudinal comparisons. Further application of DNA sequencing-based subtyping methods to the surveillance of *C. jejuni* infection will lead to significant improvements in our understanding of the epidemiology of this important pathogen.

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