JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 4221–4224 Vol. 43, No. 8
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.8.4221–4224.2005

Genetic Diversity of *Campylobacter* sp. Isolates from Retail Chicken Products and Humans with Gastroenteritis in Central Michigan

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Received 28 February 2005/Returned for modification 17 April 2005/Accepted 24 April 2005

Multilocus sequencing was used to compare *Campylobacter* sp. strains isolated from retail chicken products and humans with gastroenteritis in central Michigan. Sequence comparisons demonstrated overlapping diversity between chicken and human isolates. *Campylobacter jejuni* isolates from clinical sources had a greater diversity of flagellin alleles and a higher rate of quinolone resistance than isolates from retail chicken products.

Campylobacter species are a frequent cause of foodborne illnesses in the United States (1). Because *Campylobacter jejuni* and *Campylobacter coli* isolates occur in high numbers in the gastrointestinal tracts of chickens (18) and can be readily recovered from retail chicken products (7, 8), sporadic human infection is thought to arise most commonly via contaminated poultry products (15). To examine this link, we cultured for *Campylobacter* isolates from retail chicken products from 12 supermarkets in the Lansing, Michigan, area by using selective plates and filter enrichment techniques. Twenty-three *Campylobacter* isolates were recovered from 113 chicken samples over an 11-week period (Fig. 1). At the same time, 19 *Campylobacter* isolates from patients with gastroenteritis were obtained from the clinical laboratory of a private hospital in Lansing.

DNA was extracted from all *Campylobacter* isolates. *C. jejuni* and *C. coli* were identified by amplification of the *flaA* gene (16) and discriminated by PCR assays targeting the hippuricase (*N*-acyl-L-amino-acid amidohydrolase) gene (*hipO*) of *C. jejuni* and a *C. coli*-specific chromosomal sequence (10). Thirteen chicken isolates were *C. jejuni* isolates (56.5%) and 10 were *C. coli* isolates (43.5%), whereas 17 human isolates were *C. jejuni* isolates (89.5%) and 2 were *C. coli* isolates (10.5%). Compared to the isolation of these two species from retail chicken products, *C. jejuni* was significantly more likely to be isolated from human clinical specimens than *C. coli* ($P = 0.015$; χ^2 test).

To determine the clonal relatedness of strains, we used multilocus sequence typing (MLST) devised for *C. jejuni* (4, 13, 19) as a tool for epidemiologic investigation (3). Sequence analysis of seven housekeeping genes (4) identified, on average, 15 polymorphic nucleotide sites per locus for the 30 *C. jejuni* strains from both clinical and retail chicken product samples (Table 1). There was substantial overlap between the alleles found in strains recovered from chickens and those from patients. No alleles were specific to either source; shared alleles were found for all seven genes (Table 1). In terms of the multilocus genotypes, there were 21 sequence types (STs) resolved, 4 (19%) of which were identified in both human and chicken isolates.

The average pairwise sequence divergence of the MLST loci among the human isolates (~1%) was the same as among chicken isolates. The net genetic distance between chicken and human isolates combined was virtually zero, providing no evidence for genetic differentiation between poultry and human clinical isolates. Seventeen of the 30 (57%) *C. jejuni* strains belonged to previously recognized STs (Table 2). The remaining 13 isolates represent nine new sequence types. ST-21, ST-475, ST-918, and ST-937 were represented by both human and chicken isolates. Thirteen (43%) isolates belonged to novel STs, suggesting that unique STs circulate in local geographic regions (5).

Sixteen *C. jejuni* strains isolated here grouped into the clonal complexes ST-21, ST-48, and ST-353. Along with 1 strain each in the complexes ST-52 and ST-49, these 18 strains (60% of the *C. jejuni* isolates) fell into 1 of 17 clonal complexes that encompassed 92% of 814 *C. jejuni* strains, forming a diverse collection mainly from the United Kingdom and The Netherlands (3).

To further quantify the genetic diversity of *C. jejuni*, we examined sequence variation in two common traits used for *Campylobacter* subtyping. First, we examined allelic variation in hippuricase by designing primers (hipO-F156, 5′-AAT AGG AAA AAC AGG CGT TG-3′; hipO-R721, 5′-GTC CTG CAT TAA AAG CTC CT-3′) to amplify a 566-base-pair region in *hipO*. Sequencing of a 377-base-pair internal region of this amplicon revealed 17 polymorphic sites and 14 distinct *hipO* alleles among the 30 *C. jejuni* isolates with nucleotide variation comparable to that of the seven MLST loci (Table 1). A dendrogram constructed by combining the seven MLST and *hipO* sequences (the MLST sequences were submitted to the PubMLST *Campylobacter* database [http://campylobacter.mlst

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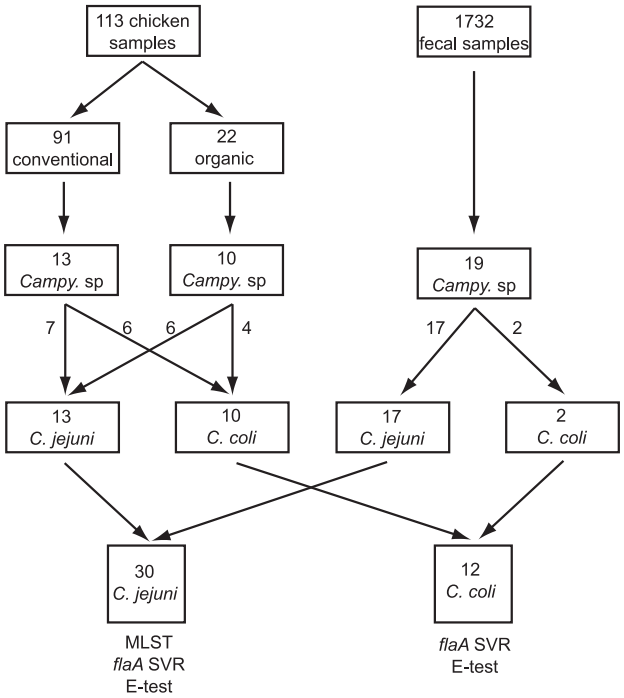

FIG. 1. Isolation and characterization of *Campylobacter* from retail chicken samples and human clinical fecal specimens. One hundred thirteen samples of retail chicken products and 1,732 human fecal samples were cultured for *Campylobacter* species (*Campy. sp.*) by use of selective media. The chicken samples came from both organically raised and conventionally raised poultry. *Campylobacter* isolates were identified to the species level by a panel of biochemical and molecular techniques as *C. jejuni* and *C. coli*. Both *C. jejuni* and *C. coli* isolates were analyzed by determination of quinolone susceptibility by Etest and determination of the sequence of the *flaA* short variable region.

.net]) demonstrates overlapping relatedness and diversity of chicken and human strains (Fig. 2).

Second, we examined sequence variation in the *flaA* short variable region (SVR) of the *C. jejuni* isolates (14) which identified 82 variable sites, 21 sequence variants (Table 1), and 10 FlaA amino acid sequence variants (Fig. 2). Assigning these sequences to STs, human strains fell into all 10 FlaA STs, while chicken isolates fell into 5 STs, with 8 of 13 in ST-3. The average divergence ± standard error of FlaA among the human isolates (7.6% ± 0.9%) was greater than that of FlaA among chicken isolates (2.2% ± 0.5%). Furthermore, the nonsynonymous base substitution-to-synonymous base substitution ratio for the *flaA* SVR of the human isolates was 0.177, compared to 0.072 for chicken isolates. The nonsynonymous base substitution-to-synonymous base substitution ratio for all seven MLST loci and *hipO* was 0.071. Given the overlapping diversity between human and chicken isolates as measured by MLST, increased flagellar diversity may reflect either reduced functional constraints on the protein or increased selection pressure within the human host.

All *Campylobacter* isolates were tested for sensitivity to ciprofloxacin by Etest (11). Two human *C. coli* isolates and one chicken isolate were ciprofloxacin resistant. Seven *C. jejuni* isolates (all from humans) representing 41.2% of human isolates, were ciprofloxacin resistant (Fig. 2). The difference between the rate of ciprofloxacin resistance encountered in chicken and human isolates was statistically significant ($P = 0.002$; χ^2 test).

A portion of the *gyrA* gene was sequenced for each isolate (17). Nine of 10 ciprofloxacin-resistant *Campylobacter* strains had a C-to-T transition that codes for the substitution of iso-

TABLE 1. Genetic diversity at *C. jejuni* MLST loci, *hipO*, and *flaA*

Locus	No. of bp	No. of variable sites	No. of alleles	Effective no. of alleles	No. of alleles shared
aspA	477	10	9	4.4	3
glnA	477	14	10	6	4
gltA	402	7	6	3.7	4
glyA	507	25	10	5.7	3
pgm	498	30	12	7.6	5
tkt	459	15	7	3.4	4
uncA	489	4	4	2.7	3
hipO	377	17	14	8.7	4
flaA	321	82	21	16.1	2
MLST average[a]	472.7	15	8.3	4.8	3.7
Overall average[b]	445.2	22.7	10.3	6.5	3.6

[a] Average values for the first seven loci, which were those used for the initial MLST analysis.
[b] Average values for all nine loci, including *hipO* and *flaA*.

TABLE 2. Multilocus sequence typing analysis of *C. jejuni* isolates

Isolate[a]	Allele no. for indicated gene[b]							Sequence type[b,c]	Clonal complex[c]
	aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*		
Chx-02	7	17	5	2	**156**	3	6	**937**	353
Hum-13	7	17	5	2	**156**	3	6	**937**	353
Chx-06	7	2	5	2	**156**	3	6	**939**	353
Hum-07	9	112	5	2	11	3	6	**936**	353
Hum-15	8	**113**	5	**121**	10	25	6	**1048**	353
Chx-09	7	17	5	2	10	3	6	353	353
Chx-04	2	1	1	3	2	1	5	21	21
Hum-17	2	1	1	3	2	1	5	21	21
Chx-17	2	1	1	3	2	1	5	21	21
Hum-14	2	1	12	3	2	1	5	50	21
Hum-10	2	1	1	3	140	3	5	806	21
Hum-03	2	4	1	2	7	1	5	48	48
Chx-03	2	4	1	4	19	62	5	475	48
Hum-01	2	4	1	4	19	62	5	475	48
Hum-04	2	4	1	4	19	1	5	918	48
Chx-10	2	4	1	4	19	1	5	918	48
Chx-05	24	30	2	2	1	59	6	**923**	460
Chx-18	24	30	2	2	1	59	6	**923**	460
Chx-22	4	7	10	4	1	7	1	45	45
Hum-18	4	7	10	4	42	7	1	137	45
Hum-05	3	1	5	84	11	11	6	467	49
Hum-02	9	25	2	10	22	3	6	52	52
Hum-08	62	4	5	2	2	1	5	572	206
Hum-06	7	17	2	15	23	3	12	51	443
Hum-09	1	1	2	83	2	3	6	**922**	Unk
Hum-12	1	1	2	83	2	3	6	**922**	Unk
Chx-11	7	2	5	53	11	3	1	**924**	Unk
Chx-21	7	2	5	53	11	3	1	**924**	Unk
Hum-19	7	84	1	10	**157**	1	6	**938**	Unk
Chx-07	2	4	27	**122**	11	3	5	**940**	Unk

[a] Chicken (Chx) and human (Hum) isolates of *C. jejuni* were subjected to MLST analysis.
[b] Boldface indicates alleles and STs first described in this study.
[c] Sequence types and clonal complexes were assigned using the PubMLST database. Unk, unknown.

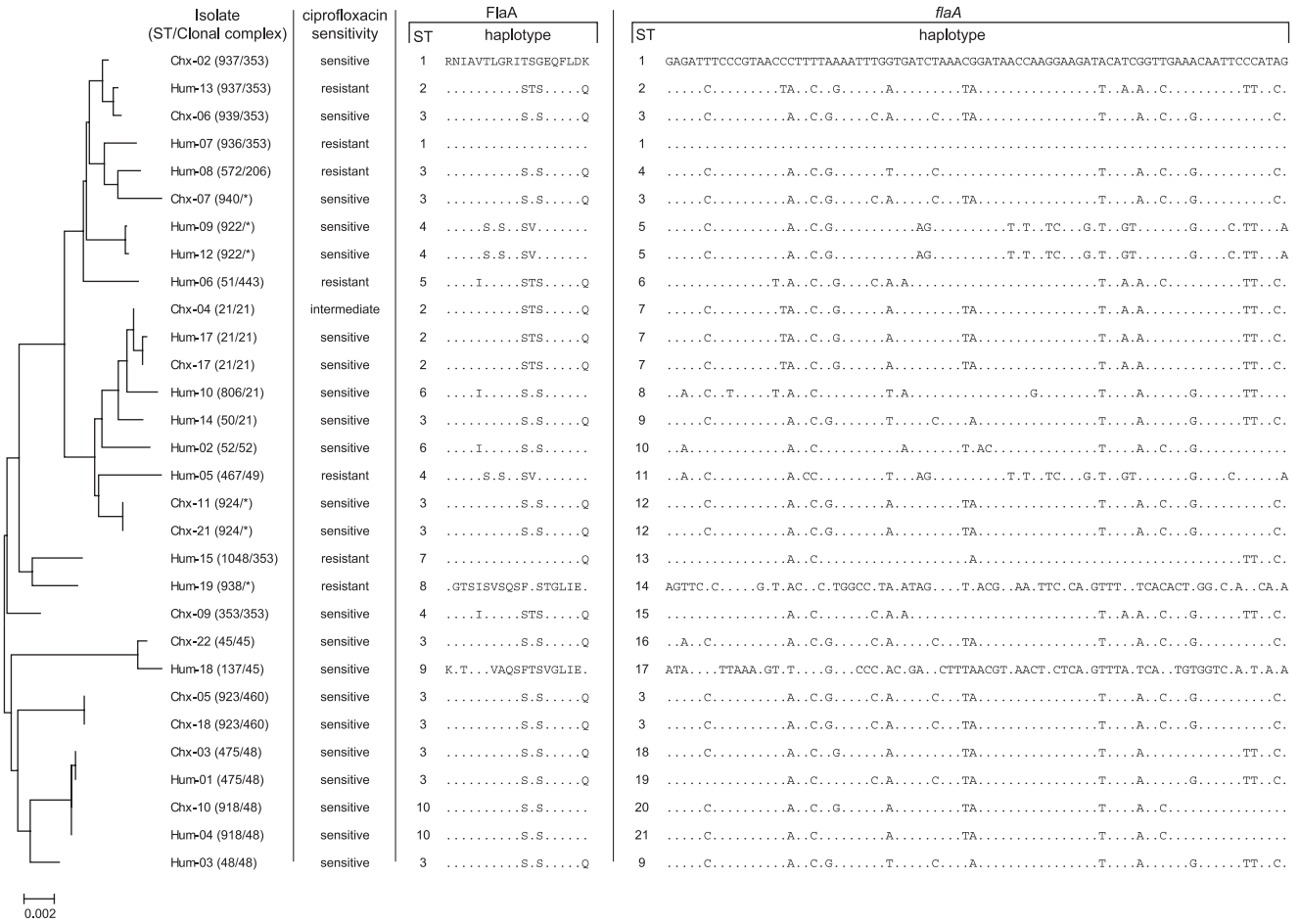

FIG. 2. Relationships between *C. jejuni* isolates from retail chicken products (Chx isolates) and from humans with gastroenteritis (Hum isolates) as determined by MLST, antibiotic resistance typing, and flagellin gene short variable region typing. A phylogeny was constructed by concatenating the seven MLST loci and the *hipO* locus. ST and clonal complex assignment were performed using only the seven MLST loci. Ciprofloxacin sensitivity was determined by the use of the Etest. Amino acid (FlaA) and nucleotide (*flaA*) haplotypes (listing of variable sites) are shown for each isolate. STs for FlaA and *flaA* were assigned in the order of discovery. The dendrogram was constructed using the program MEGA2 (9a).

leucine for threonine at position 86 (T86I), the most common mutation found in highly fluoroquinolone-resistant *Campylobacter* strains (21).

Geographically linked *C. jejuni* isolates from poultry and humans can have corresponding high rates of quinolone resistance (9, 12). Among our *C. jejuni* isolates in this study, unexpectedly, none of the chicken isolates was ciprofloxacin resistant, while 7 of the 17 clinical isolates of *C. jejuni* were ciprofloxacin resistant, in all cases associated with the T86I *gyrA* amino acid substitution. The treatment of patients with quinolones could select for resistant isolates and drive them to high frequency. Quinolone resistance (via the T86I mutation) can arise rapidly in *C. jejuni* and *C. coli* in the gastrointestinal tracts of animals and humans treated with quinolones (2, 6, 20).

In summary, a polyphasic scheme was used to compare the diversity of *Campylobacter* species isolated from retail poultry and humans with gastroenteritis. MLST analysis of housekeeping genes of *C. jejuni* strains provides support for the hypothesis that retail chicken products can serve as food reservoirs for *C. jejuni* that leads to human gastroenteritis. Analysis of potentially variable traits (flagellar typing and antibiotic resistance profiling) suggests that additional selection occurs at some point during the transition from the environmental reservoir and sampling of the human host, perhaps within the gastrointestinal tract of the host itself.

Nucleotide sequence accession numbers. *flaA* SVR sequences were submitted to GenBank under the accession numbers AY927807 to AY927484, and the partial *hipO* sequences were submitted under the accession numbers AY944146 to AY944175.

Parts of this study were funded with support from the Michigan State University Center for Emerging Infectious Diseases.

We thank Jennifer Ellis-Cortez for expert technical assistance.

REFERENCES

1. **Centers for Disease Control and Prevention.** 2003. Preliminary FoodNet data on the incidence of foodborne illnesses–selected sites, United States, 2002. Morb. Mortal. Wkly. Rep. **52:**340–343.
2. **Delsol, A. A., J. Sunderland, M. J. Woodward, L. Pumbwe, L. J. Piddock, and J. M. Roe.** 2004. Emergence of fluoroquinolone resistance in the native

Campylobacter coli population of pigs exposed to enrofloxacin. J. Antimicrob. Chemother. **53:**872–874.

3. **Dingle, K. E., F. M. Colles, R. Ure, J. A. Wagenaar, B. Duim, F. J. Bolton, A. J. Fox, D. R. Wareing, and M. C. Maiden.** 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg. Infect. Dis. **8:**949–955.
4. **Dingle, K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. Willems, R. Urwin, and M. C. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39:**14–23.
5. **Duim, B., P. C. Godschalk, N. van den Braak, K. E. Dingle, J. R. Dijkstra, E. Leyde, J. van der Plas, F. M. Colles, H. P. Endtz, J. A. Wagenaar, M. C. Maiden, and A. van Belkum.** 2003. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curaçao, Netherlands Antilles. J. Clin. Microbiol. **41:**5593–5597.
6. **Engberg, J., J. Neimann, E. M. Nielsen, F. M. Aerestrup, and V. Fussing.** 2004. Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. Emerg. Infect. Dis. **10:**1056–1063.
7. **Ge, B., D. G. White, P. F. McDermott, W. Girard, S. Zhao, S. Hubert, and J. Meng.** 2003. Antimicrobial-resistant *Campylobacter* species from retail raw meats. Appl. Environ. Microbiol. **69:**3005–3007.
8. **Hanninen, M. L., P. Perko-Makela, A. Pitkala, and H. Rautelin.** 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin. Microbiol. **38:**1998–2000.
9. **Hein, I., C. Schneck, M. Knogler, G. Feierl, P. Plesss, J. Kofer, R. Achmann, and M. Wagner.** 2003. *Campylobacter jejuni* isolated from poultry and humans in Styria, Austria: epidemiology and ciprofloxacin resistance. Epidemiol. Infect. **130:**377–386.

9a. **Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei.** 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics **17:**1244–1245.

10. **Linton, D., A. J. Lawson, R. J. Owen, and J. Stanley.** 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J. Clin. Microbiol. **35:**2568–2572.
11. **Luber, P., E. Bartelt, E. Genschow, J. Wagner, and H. Hahn.** 2003. Comparison of broth microdilution, E test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol. **41:**1062–1068.
12. **Luber, P., J. Wagner, H. Hahn, and E. Bartelt.** 2003. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001–2002 from poultry and humans in Berlin, Germany. Antimicrob. Agents Chemother. **47:**3825–3830.
13. **Manning, G., C. G. Dowson, M. C. Bagnall, I. H. Ahmed, M. West, and D. G. Newell.** 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl. Environ. Microbiol. **69:**6370–6379.
14. **Meinersmann, R. J., L. O. Helsel, P. I. Fields, and K. L. Hiett.** 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. J. Clin. Microbiol. **35:**2810–2814.
15. **Neimann, J., J. Engberg, K. Molbak, and H. C. Wegener.** 2003. A case-control study of risk factors for sporadic campylobacter infections in Denmark. Epidemiol. Infect. **130:**353–366.
16. **Oyofo, B. A., S. A. Thornton, D. H. Burr, T. J. Trust, O. R. Pavlovskis, and P. Guerry.** 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. J. Clin. Microbiol. **30:**2613–2619.
17. **Piddock, L. J., V. Ricci, L. Pumbwe, M. J. Everett, and D. J. Griggs.** 2003. Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. J. Antimicrob. Chemother. **51:**19–26.
18. **Rosef, O., G. Kapperud, S. Lauwers, and B. Gondrosen.** 1985. Serotyping of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from domestic and wild animals. Appl. Environ. Microbiol. **49:**1507–1510.
19. **Suerbaum, S., M. Lohrengel, A. Sonnevend, F. Ruberg, and M. Kist.** 2001. Allelic diversity and recombination in *Campylobacter jejuni*. J. Bacteriol. **183:**2553–2559.
20. **van Boven, M., K. T. Veldman, M. C. de Jong, and D. J. Mevius.** 2003. Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. J. Antimicrob. Chemother. **52:**719–723.
21. **Zirnstein, G., L. Helsel, Y. Li, B. Swaminathan, and J. Besser.** 2000. Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiol. Lett. **190:**1–7.