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Stability of Related Human and Chicken *Campylobacter jejuni* Genotypes after Passage through Chick Intestine Studied by Pulsed-Field Gel Electrophoresis

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The genomic stability of 12 *Campylobacter jejuni* strains consisting of two groups of human and chicken isolates was studied by analysis of their PFGE (pulsed-field gel electrophoresis) patterns after passage through newly hatched chicks' intestines. The patterns of *Sma*I, *Sal*I, and *Sac*II digests remained stable after intestinal passage, except for those of two strains. One originally human strain, FB 6371, changed its genotype from II/A (*Sma*I/*Sac*II) to I/B. Another strain, BTI, originally isolated from a chicken, changed its genotype from I/B to a new genotype. The genomic instability of the strains was further confirmed by *Sal*I digestion and ribotyping of the *Hae*III digests. In addition, heat-stable serotype 57 of strain FB 6371 changed to serotype 27 in all isolates with new genotypes but remained unchanged in an isolate with the original genotype. Serotype 27 of strain BTI remained stable. Our study suggests that during intestinal colonization, genomic rearrangement, as demonstrated by changed PFGE and ribopatterns, may occur.

Typing has been widely used in the characterization of *Campylobacter jejuni* isolates from different sources, such as human diarrheal stools, animal fecal samples, and food and water samples (14). The conventional typing systems available are serotyping (11, 17), biotyping (12), and phage typing (19). Molecular typing methods, including ribotyping, RFLP (restriction fragment length polymorphism) of the *fla* gene, and PFGE (pulsed-field gel electrophoresis), have been used during the 1990s. Because *C. jejuni* has only three copies of the ribosomal genes, ribotyping is not a very good method for distinguishing them and has not achieved wide use (6). The flagellin locus has been sequenced and analyzed, showing that intragenetic and intergenetic recombination in this region of the genome is rather common and may lead to variability in genotypes and produce difficulties in the interpretation of genotyping results (9). Genetic recombination of flagellar genes detected by PCR-RFLP is not correlated with the serotype conversions occasionally seen in chicken flocks (1, 3). Several studies have revealed PFGE to be a very good method for distinguishing subtypes within serotypes (4, 23) and is also useful for typing of strains that are untypeable with antisera (4). The discriminatory power of PFGE typing can be increased if two enzymes, for example, *Sma*I and *Kpn*I (4) or *Sac*II (7) are used in combination. The stability of PFGE patterns is unknown, although some evidence exists that patterns might change in stressful environments (26).

During our longitudinal studies on the epidemiology of human campylobacter infections in Finland in 1995 and 1996, we identified two groups of *C. jejuni* strains with identical or highly related *Sma*I patterns and differing *Sac*II patterns which shared many fragments (7). Our hypothesis was that under various environmental conditions, these patterns might be transformed. In the present study, the genomic stability of these two *C. jejuni* PFGE genotype groups was studied after passage through chick intestines. The first group included seven strains with identical (pattern I) or related (pattern II) *Sma*I patterns and with *Sac*II patterns which differed by 4 to 11 fragments and *Sal*I patterns with 2 to 4 differing fragments. The second group consisted of five strains with *Sma*I PFGE pattern XI, *Sac*II patterns differing by 7 to 11 fragments, and *Sal*I patterns differing by 1 to 4 fragments. Combined *Sma*I/*Sac*II/*Sal*I PFGE patterns were designated genotypes. Since their isolation, the strains were stored deep-frozen at −70°C in sterile skim milk with 15% glycerol. The strains were grown on brucella (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) blood agar plates for 2 days and in brucella broth for 1 day in a microaerobic atmosphere at 37°C.

Brucella broth cultures were inoculated by oral gavage into the crops of newly hatched chicks (inoculum size, 10^8 to 10^9 CFU per animal). Each strain was inoculated into seven animals. Ten control animals were used to verify that chicks did not have campylobacters before the inoculation experiment by culturing of cecal samples. Each group of chicks was reared in a separate solid-bottom cardboard box at the Laboratory for Animal Activities for 6 days. After 6 days, the animals were euthanized with CO_2 and campylobacter CFUs in cecal samples diluted in 0.1% peptone water were counted and 0.1 volumes of 10^{-5} to 10^{-8} dilutions were spread on modified charcoal cefoperazone deoxycholate agar (Oxoid) medium. All modified charcoal cefoperazone deoxycholate agar plates were incubated microaerobically at 42°C. Four colonies were subcultured from a 10^{-6} or 10^{-8} dilution from each chick and confirmed by colony morphology, gram staining, and positive catalase and hippurate tests to be *C. jejuni*. Two colonies from each chick were used for PFGE pattern analysis.

For PFGE analysis, the isolates were grown on brucella blood agar for 2 days at 37°C in a microaerobic atmosphere. The bacterial cells were harvested and treated with formaldehyde to inactivate endogenous nuclease (5). Otherwise, DNA was prepared by the method of Maslow et al. (13) as described earlier (7). The DNA fragments were separated with Gene

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TABLE 1. Serotypes, PFGE patterns, and ribotypes of *C. jejuni* strains used for studies on genomic stability

Group (*Sma*I pattern) and strain	Serotype	PFGE pattern		Fig. 1 lane no.	Ribotype (*Hae*III)
		*Sac*II	*Sal*I		
1 (I/II)					
Human strains					
FB 6032	27	B	i	1	1
FB 6371[a]	57	A	ii	2	2
FB 5194	6,7	J	i	6	1
FB 5592	55	K	i	7	3
FB 6021	41	L	iii	9	4
Chicken strains					
50A	12	E	i	4	3
BTI	27	B	i	8	1
2 (XI)					
Human strains					
FB 5025	21	S	iv		2
FB 6362	21	R	iv		5
Chicken strains					
Ci-5	ND[b]	T	iv		6
40A	6,7	U	iv		7
36A	ND	Q	iv		7

[a] *Sma*I pattern II.
[b] ND, not done.

Navigator (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) in 1% agarose gel in 0.5× TBE (45 mmol of Tris, 45 mmol of boric acid, 1 mmol of EDTA) at 200 V. *Sma*I and *Sal*I fragments were separated with a ramped pulse of 0.5 to 25 s for 20 h, and *Sac*II fragments were separated with a ramped pulse of 0.3 to 20 s for 20 h.

All 12 strains and the isolates of FB 6371 and BTI with changed PFGE patterns after intestinal passage were ribotyped after digestion of their DNAs with *Hae*III. *Hae*III digests were made both from the respective PFGE plugs and from DNA isolated from bacterial cells after microaerobic growth in brucella broth at 37°C for 44 h. DNA was isolated and ribotyping was performed as described earlier (6). Heat-stable Penner's serotyping of the 12 strains and of the selected isolates after passage was performed as described earlier (18).

The selected *C. jejuni* strains and their sources of isolation, serotypes, PFGE patterns, and ribotypes are shown in Table 1. Figure 1a, b, and c shows the *Sma*I, *Sal*I, and *Sac*II patterns of certain of the *Sma*I pattern type I strains (strain FB 7052 in lane 3 and strain 48A in lane 5 were not included in the stability studies), respectively. *Sal*I patterns show some variation (Fig. 1b, lanes 1, 2, 7, 8, and 10). *Sac*II, which cuts the DNA most frequently, generated differing patterns with many shared fragments (Fig. 1c). Patterns of *Sma*I pattern type XI strains are not shown.

None of the control chicks was colonized with campylobacters. All chicks were from the same breeder never known to have campylobacters. All animals were colonized with all of the selected PFGE genotypes. Campylobacter counts were 10^8 to 10^{10} CFU/g. PFGE pattern analysis was performed with 14 colonies of each strain isolated from seven chicks, two from each. All genotypes of the *Sma*I pattern XI group remained unchanged after passage (results not shown). Similarly, all genotypes of the *Sma*I pattern type I strains remained unchanged, except that genotype II/A (strain FB 6371) changed

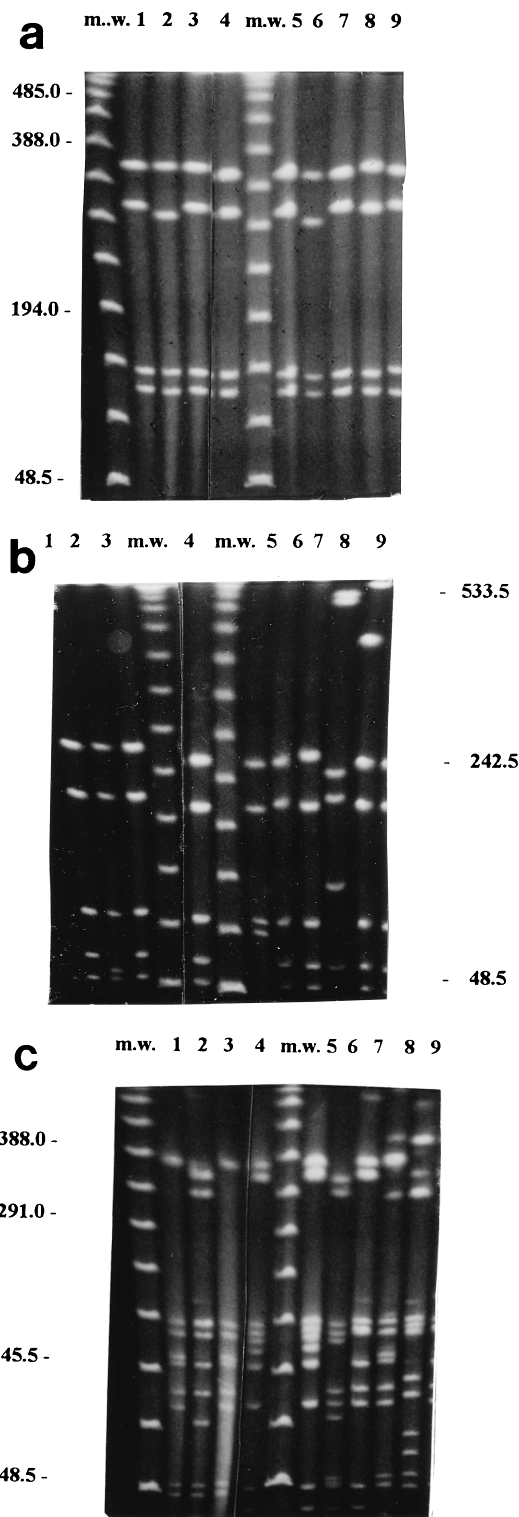

FIG. 1. *Sma*I (a), *Sal*I (b), and *Sac*II (c) patterns of *C. jejuni* strains with highly similar *Sma*I pattern I or II. Lanes: 1, strain FB 6032 (genotype I/B), 2, FB 6371 (II/A); 3, FB 7052 (I/B); 4, 50A (I/E); 5, 48A (I/E); 6, FB 5194 (I/J); 7, FB 5592 (I/K); 8, FB 5622 (I/B); 9, FB 6021 (I/L). Molecular size marker, 48.5-kb lambda concatemer (lanes m.w.).

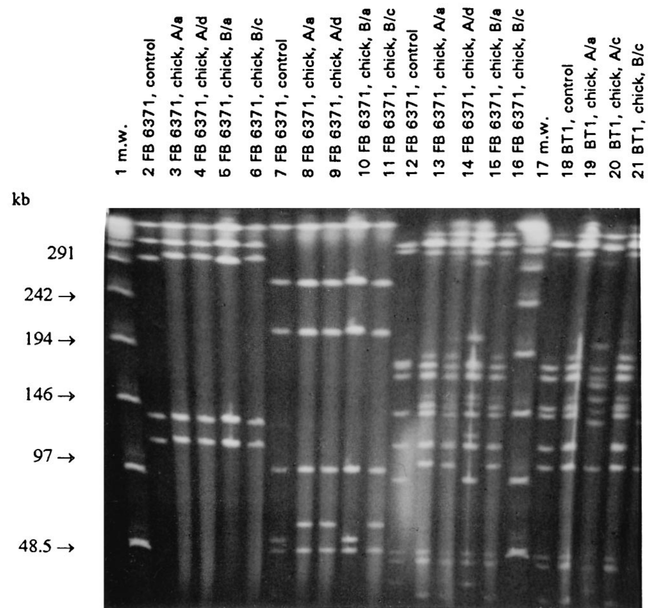

FIG. 2. Patterns of *C. jejuni* FB 6371 and BTI after passage through chicks' intestines. Lanes: 2, 7, and 12, *Sma*I, *Sal*I, and *Sac*II patterns of strain FB 6371, respectively, before the colonization study; 3 to 6 (*Sma*I), 8 to 11 (*Sal*I), and 13 to 16 (*Sac*II), patterns of four colonies of strain FB 6371 isolated from chicken fecal samples after the colonization experiment. Changed patterns of three colonies A/a, A/d, and B/c, are seen in lanes 8, 9, and 11 and 13, 14, and 16. Similarly, the control *Sac*II pattern of strain BTI is shown in lane 18, the unchanged patterns are in lanes 19 and 20, and the changed pattern is in lane 20. Molecular size markers (48.5-kb lambda concatemer) are shown in lanes 1 and 17.

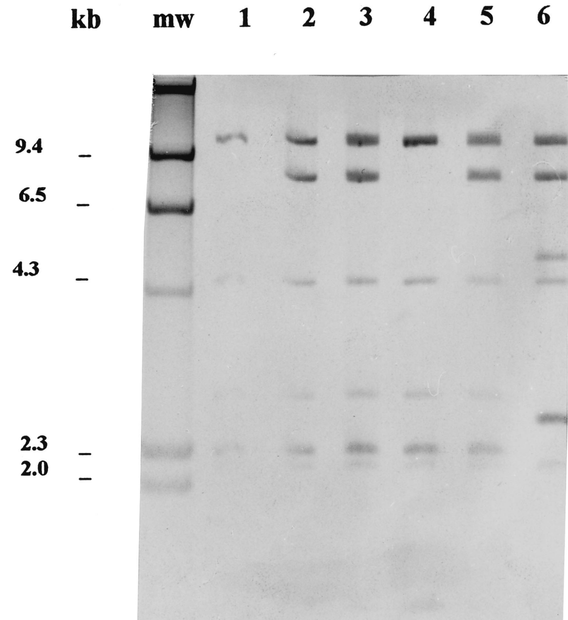

FIG. 3. Ribotypes (*Hae*III digests) of selected isolates of strains FB 6371 and BTI before and after colonization in chicks' intestines. Lanes: 1, strain FB 6371 before inoculation, ribotype 2; 2 and 3, changed ribotype 1 of two colonies (chicks A/a and A/d) of strain FB 6371 after colonization; 4, colony (chick B/a) with unchanged ribotype 2; 5, strain BTI before inoculation, ribotype 1; 6, isolate (BTI, chick A/c) after colonization, ribotype 8; mw, molecular size marker (Marker II; Boehringer Mannheim, Mannheim, Germany).

into genotype I/B in all seven chicks; only one of the 14 colonies studied had the original pattern, II/A. Figure 2 shows the *Sma*I (lanes 2 to 6), *Sal*I (lanes 7 to 11), and *Sac*II (lanes 12 to 16) patterns of four isolates representing the original genotype (lanes 2, 7, and 12), the stable pattern (lanes 5, 10, and 15, and the changed pattern (lanes 3, 4, 6, 8, 9, 11, 13, 14, and 16). Six fragments changed in the *Sac*II pattern. One of the 14 colonies (strain BTI) changed from pattern I/B to a new *Sac*II pattern type (Fig. 2, lane 20). Change was also visible in the location of one fragment of the *Sma*I and *Sal*I patterns (results not shown). Ribotyping of *Hae*III digests of the selected FB 6371 and BTI isolates showed that when PFGE pattern II/A was changed to pattern I/B, the respective change was seen in the ribopatterns as well (Fig. 3, lanes 1 to 4). Similarly, when PFGE pattern I/B of strain BTI was changed, the ribopattern was also changed (Fig. 3, lanes 5 and 6). Passaged isolates with the changed genotype of strain FB 6371 changed from serotype 57 to serotype 27. The serotype of the isolate with the unchanged genotype remained 57. The serotype, 27, of strain BTI remained the same in isolates with unchanged and changed genotypes.

During the last 10 years, molecular subtyping systems have been developed and extensively used to support studies on the epidemiology of microbial infections (1, 7, 16, 22, 25). Several methods are based on the analysis of RFLP of a single locus or on entire-genome macrorestriction analysis (PFGE). Information on the stability of the patterns over a short (during an epidemic) or long (years, decades) time span or during in vitro culture and storage is required for evaluation of the performance characteristics of the methods (22). Our results suggest that intestinal colonization may favor genetic recombination (rearrangement, insertions, deletions, point mutations) in *C. jejuni* which will become visible in the PFGE patterns and in ribopatterns, because in 2 of our 12 strains such variability was evident. Recently, Wassenaar et al. (26) studied PFGE genotype distribution in poultry meat batches and found variable, related *C. jejuni* PFGE genotypes; they suggested that this variation might have resulted from genetic recombination occurring under stressful conditions during meat storage. However, when a *C. jejuni* genotype was passaged in two day-old chicks, the genotype remained stable. On the contrary, in our study, we were able to find changed patterns in 2 of 12 strains, suggesting that either the method used was not sensitive enough to reveal genetic variations in all of the strains at different rates or that the genotypes differ in genomic stability. PFGE is a rather laborious and expensive method, and the number of colonies which can be analyzed is limited. In our study, both the number of strains and that of the chicks used for in vivo passage were higher than in the studies of Wassenaar et al. (26), thus increasing the possibility that we would detect genomic instability. We were able to show simultaneous change in the genotype and in the serotype. Recent studies have revealed that intergenomic and intragenomic recombinations between *flaA* and *flaB* genes is evident (9), suggesting that this genomic region is unstable in *C. jejuni*.

The strains in our study were selected from a large collection of human and chicken strains with a wide variety of PFGE patterns (7). *Sma*I pattern I strains formed the predominant group among human domestic campylobacter strains in 1996 or were isolated from chickens during the same time period. Pattern II/A is uncommon among Finnish strains, and it has been identified in four strains in 1996 to 1998 (unpublished results). *Sma*I pattern XI strains were either human isolates from two small outbreaks (patterns XI/S and XI/R) or chicken isolates (patterns XI/Q, XI/U, and XI/T). *C. jejuni* is highly

adapted for growth in the intestines of chickens, and if a flock is colonized with *C. jejuni*, most of the animals acquire the organism and excrete it in high numbers, i.e., 10^6 to 10^8 CFU/g of fecal material (2, 10). Several studies have shown that usually only one or a few serotypes or genotypes colonize a flock (1, 2, 10, 21). Thus, no direct evidence of natural genetic instability within commercially reared flocks is available. If several genotypes have been identified in a flock, this has been explained by several contamination sources (21). Ayling et al. (1) performed a longitudinal study of 96 broiler houses by use of PCR-RFLP analysis of *flaA* and *flaB* genes and found that, in most cases, one genotype was predominant, suggesting genotype stability during the rearing of a flock. Detection of genomic instability, however, depends on the number of samples taken and the methods used. PFGE has been shown to be a more distinguishing typing method than PCR-RFLP for flagellar genes (4). Although we did find genomic instability in genotypes I/B and II/A, we could not identify this phenomenon in the other genotypes or in the second strain of genotype I/B. Ribotyping of isolates with changed genotypes indicated that during intestinal colonization, new restriction sites were also formed in ribosomal regions. In serotype 57 strain 6371, serotype conversion was seen in addition to genetic change.

Genotype I/B was isolated for the first time 10 years ago from fecal samples of cattle (8), suggesting long-term stability of this genotype. We have also identified one of the genotypes, I/E, in chickens over at least a 3-year period, also indicating the stability of this genotype (unpublished results). Steinbrueckner et al. (20) found that the PFGE patterns of the strains of 48 campylobacter patients remained stable in stool samples studied at a mean interval of 3 weeks. However, in one patient, the first and second isolates exhibited a difference of four bands in their PFGE patterns. It was not clear if this patient had two infecting strains or if the original pattern had changed during the infection (20).

All of the common typing methods suggest that *C. jejuni* is a heterogeneous organism (6, 7, 16, 17, 19). This heterogeneity may also result from genomic instability. Genomic plasticity may thus be an important characteristic for survival in various hosts known to be colonized by *C. jejuni* (24). More data on the genetic stability of PFGE patterns and other genotypes is needed.

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