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DNA Relatedness Among Strains of *Campylobacter jejuni* and *Campylobacter coli* with Divergent Serogroup and Hippurate Reactions

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Eleven strains of *Campylobacter* from earlier fluorescent-antibody studies were examined by DNA hybridization to determine their species. Three of the strains hydrolyzed sodium hippurate, and eight did not. Four of the hippurate-negative strains were in *Campylobacter jejuni* serogroups, and the remaining strains were in both *C. jejuni* and *Campylobacter coli* serogroups. DNA relatedness to type strains of *C. jejuni* and *C. coli* indicated that all three of the hippurate-positive strains and two of the hippurate-negative strains were *C. jejuni*. The six remaining hippurate-negative strains were *C. coli*. Two of the hippurate-negative strains in *C. jejuni* serogroups were *C. jejuni*, and two were *C. coli*. Three of the strains in serogroups of both species were *C. jejuni*, and four were *C. coli*. These studies confirm that a few strains of *C. jejuni* are hippurate negative and show that identical or highly related antigens are found in *C. coli* and *C. jejuni*.

In previous studies, we developed immunofluorescence reagents for *Campylobacter* species which defined 17 direct fluorescent-antibody (FA) serogroups of *Campylobacter jejuni*, three direct FA serogroups of *Campylobacter coli*, and two serogroups of *Campylobacter fetus* (4, 5). The immunogens for the *C. jejuni* and *C. coli* immunoglobulins were identified by their DNA relatedness at the species level to the type strains of *C. jejuni* and *C. coli*. Of the 308 strains that were groupable with these direct FA reagents for *C. jejuni* and *C. coli*, 267 hippurate-positive strains were in *C. jejuni* serogroups, 29 hippurate-negative strains were in *C. coli* serogroups, and 12 strains had conflicting serogroup and hippurate reactions. Each of our previous reports (4, 5) included discussion of these 12 strains. One strain (E8343) has been lost, but the other 11 strains have now been examined by DNA hybridization to genetically determine their species, and the results of those studies are presented in this report.

These strains were not subjected to closer scrutiny because of variable hippurate reactions or any other questionable biochemical or morphological characteristic. They were examined for DNA relatedness to determine the reason for the conflict between their FA and hippurate reactions. Repeated direct FA and hippurate tests gave reproducible but conflicting results, and we needed to determine whether the serological or biochemical data were aberrant.

Although each of the 11 strains had been exhaustively tested during the previous studies, each was again tested, and all test results were confirmed before growth was harvested for the DNA extractions. All strains were grown on fresh plates of blood agar (heart infusion agar containing 5% defibrinated rabbit blood) for 18 to 24 h at 35°C in approximately 5% oxygen as previously described (3). Strains were tested for their ability to hydrolyze sodium hippurate by the rapid tube method of Hwang and Ederer (4, 6) and were examined by direct FA as previously described (4, 5). The cell growth on approximately 25 blood agar plates (150 by 15 mm) of each strain was harvested for extraction and purification of DNA as previously described (1). Each of the 11 strains and two reference strains were examined in two separate experiments for DNA relatedness to the labeled type strains of *C. jejuni* and *C. coli* at the stringent incubation temperature of 65°C; the details of each of these procedures used to obtain DNA relatedness data have been described (1).

The results of the two independent analyses were averaged to obtain the DNA data shown in Table 1. Labeled DNA from the type strain of *C. jejuni* (CIP 702) showed 80 to 91% relatedness to five of the strains and only 34 to 42% relatedness to the other six strains. Labeled DNA from the type strain of *C. coli* (CIP 7080) showed only 32 to 44% relatedness to the set of five strains with high DNA relatedness to *C. jejuni* and 78 to 94% relatedness to the other six strains.

During hippurate hydrolysis tests of the five *C. jejuni* strains, one strain gave a yellow to light-gray reaction, one was a very light purple, and three were a deep black-purple each time the tubes were examined for color development. Only the dark-purple reactions were recorded as positive for hippurate hydrolysis (Table 1). Some laboratories would report the very light-purple hippurate reaction seen repeatedly with one of these strains as a positive reaction, but none would interpret the yellow-gray reaction of the other strain as positive. Our data document at least one, and possibly two more, strains of *C. jejuni* that do not hydrolyze sodium hippurate to form glycine as measured by the rapid tube test. A previous report by Harvey and Greenwood (2) discussed another such strain of *C. jejuni*. Our six strains of *C. coli* always gave a light-gray reaction and were recorded as hippurate negative. All of the hippurate-positive strains were *C. jejuni*, but two of the hippurate-negative strains were also *C. jejuni*. Our data, therefore, confirm the basic reliability of the hippurate hydrolysis test for differentiating between strains of *C. jejuni* and *C. coli*, but also confirm its fallibility. By these and previous studies (2), hippurate-positive strains are *C. jejuni*, whereas hippurate-negative strains are usually *C. coli*, but occasionally may be *C. jejuni*.

During our direct FA studies of campylobacters, 271 strains were placed in *C. jejuni* serogroups, 30 in *C. coli* serogroups, and seven in serogroups of both species (4, 5).

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TABLE 1. DNA hybridization data for strains with conflicting serology and hippurate reactions

Campylobacter strain and source of unlabeled DNA	% Relatedness of labeled DNA (relative binding ratio at 65°C)[a]		Hippurate hydrolysis	Direct FA serogroup	
	C. jejuni CIP 702	*C. coli* CIP 7080		*C. jejuni*	*C. coli*
Type strains					
C. jejuni CIP 702	100	41	+ (black-purple)	7	—[b]
C. coli CIP 7080	34	100	− (light gray)	—	C-1
C. jejuni:					
E9454	91	44	− (yellow-gray)	2	—
PC95	86	32	− (light purple)	14	—
E6162	84	32	+ (black-purple)	1	C-1
S5	80	32	+ (black-purple)	8	C-1
KC1481 (MEL)	88	38	+ (black-purple)	8	C-1
C. coli:					
F589	42	78	− (light gray)	4	—
F1753	36	78	− (light gray)	4	—
PC228	37	80	− (light gray)	3:7	C-2
KC1570 (PC66)	34	81	− (light gray)	8	C-1
KC1572 (PC67)	38	84	− (light gray)	8	C-1
F1789	39	94	− (light gray)	8	C-1

[a] Data obtained from the average of two separate experiments with each strain at the stringent temperature of 65°C. Relative binding ratio = [(percent heterologous DNA bound to hydroxyapatite)/(percent homologous DNA bound to hydroxyapatite)] × 100.
[b] —, Negative.

Four of the strains placed in *C. jejuni* serogroups were hippurate negative, and the new DNA data (Table 1) show that the two strains in *C. jejuni* serogroups 2 and 14 really are *C. jejuni*, but the two strains in *C. jejuni* serogroup 4 are *C. coli*. So, both the serogroup and hippurate reactions were unreliable for separating these four strains into species because two *C. coli* strains reacted with an immunoglobulin to *C. jejuni* and two *C. jejuni* strains were hippurate negative.

The seven strains that reacted equally with direct FA reagents of both species are shown in Table 1. By DNA relatedness, the three hippurate-positive strains were *C. jejuni* and the four hippurate-negative strains were *C. coli*, yet the *C. jejuni* strains reacted as well with the *C. coli* serogroup C-1 conjugate as they did with their respective *C. jejuni* conjugates, and the *C. coli* strains reacted as well with some *C. jejuni* conjugates as with their respective *C. coli* conjugates. All of these reactions were a 3 to 4^+ intensity at dilutions equal to the homologous titers of the conjugates. Five of the seven strains reacted with the same two conjugates, one of each species; two strains of *C. jejuni* and three strains of *C. coli* were placed in *C. jejuni* serogroup 8 and in *C. coli* serogroup C-1. The immunogen for the *C. coli* serogroup C-1 conjugate was the type strain of *C. coli*, CIP 7080.

None of the conjugates for the three *C. coli* serogroups reacted with the cells of any of the other immunogens during these studies. Two of the *C. jejuni* conjugates, however, gave 3 to 4^+ staining of a single heterologous immunogen at the concentrated 1:16 dilution but were negative at their respective working dilutions. One of these reagents was the conjugate for *C. jejuni* serogroup 8; it reacted with the *C. coli* type strain immunogen at low dilution, but not at its high working dilution of 1:512. Strains were assigned to a serogroup on the basis of 3 to 4^+ staining reactions with the working dilution of a conjugate (4).

As more direct FA serogroups are developed for *C. jejuni* and *C. coli*, more reagents may be found which react with isolates of both species through shared antigens, but except for the few cases discussed above, each species was basically characterized by a distinct set of antigens. All of our direct FA studies were done with labeled immunoglobulins from unabsorbed antisera. More specificity might be achieved with absorbed reagents, but so far it has not been necessary.

Recently, Penner et al. divided their passive hemagglutination serotyping scheme into separate schemes for serotyping *C. jejuni* and *C. coli* (7). They used the results of the hippurate hydrolysis test on the 59 serotype reference strains to distinguish between the two species and found 42 hippurate positive (*C. jejuni*) and 17 hippurate negative (*C. coli*). Of the 2,238 strains typable in their unabsorbed antisera, only 16 had conflicting serotype and hippurate reactions; 12 hippurate-negative strains reacted in *C. jejuni* antisera, and four hippurate-positive strains reacted in *C. coli* antisera. In addition, some isolates of both species reacted in one of the *C. jejuni* antisera. Their sample size was much larger than ours, but their results were quite similar.

All of these data support the use of hippurate hydrolysis and direct FA serogrouping with genetically defined reagents to differentiate between the species *C. jejuni* and *C. coli*; these data also remind us, however, to beware of overconfidence with any single test system except the one for DNA relatedness to type strains under specified conditions.

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