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Application of Serotyping and Chromosomal Restriction Endonuclease Digest Analysis in Investigating a Laboratory-Acquired Case of *Campylobacter jejuni* Enteritis

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A frequently passaged laboratory strain of *Campylobacter jejuni* was confirmed by serotyping on the basis of thermostable antigens and by bacterial chromosomal restriction endonuclease digests to be the causative agent of enteritis in a laboratory worker.

Campylobacter jejuni strains are now well known etiological agents of human enteritis (2, 3, 8). Reports of accidental infections among laboratory personnel are, however, rare, although the infective dose has been reported to be only 500 bacterial cells (7). One report describes an infection acquired by an investigator attempting to demonstrate transmission of diarrhea in puppies with two strains of *C. jejuni* (6). By bactericidal assay tests, it was found that serum antibody was produced against only one of the strains. This provided the evidence to support the conclusion that the infection was acquired as a result of exposure to *C. jejuni* in the laboratory. In the present communication, experimental evidence is presented to document the occurrence of another laboratory-acquired infection. The research in our laboratory is primarily directed towards development of serotyping schemes for *C. jejuni* and *Campylobacter coli*, and, when a member of the group acquired gastroenteritis, investigations were conducted to identify the infecting agent. Stool specimens were found positive for *C. jejuni*. A sweep of growth and 10 colonies were taken from the primary isolation medium, and thermostable antigen was extracted from each for titration in the 42 *C. jejuni* antisera that had been produced in our laboratory. The results showed that two antisera (against *C. jejuni* serotypes 8 and 17) reacted with each preparation of antigenic material. The titrations were performed using the passive (indirect) hemagglutination technique as described previously (5).

On day 3 before the onset of diarrhea, the worker had been conducting tests with *C. jejuni* reference strains for the thermostable antigen serotypes 9, 17, 19, 27, 29, 32, and 36. It appeared, therefore, that the cause of the diarrhea was exposure to serotype 17. However, it is known that cross-reactions occur between serotypes 8 and 17, and to determine whether the infecting strain was indeed serotype 17 or was due to exposure to a serotype 8 strain acquired elsewhere or under different circumstances, additional experiments were performed. Antisera against serotypes 8 and 17 were cross-absorbed and cross-titrated, and the isolate was tested in

TABLE 1. Results of serotyping with unabsorbed and cross-absorbed antisera 8 and 17

Antigens prepared from:	Unabsorbed antisera		Cross-absorbed antisera[a]		Antisera absorbed with homologous strain	
	8	17	8(17)	17(8)	8(8)	17(17)
Serotype (reference strains)						
8 (C142)	320[b]	40	160	–	–	–
17 (MK15)	320	640	–	160	–	–
Patient isolate	160	320	–	160	–	–

[a] Strains used for absorption are indicated within parentheses. Antisera (0.2 ml undiluted) were incubated for 2 h with 3.8 ml of suspension of bacteria prepared from cells from 5 plates and heated for 1 h at 100°C.

[b] Titers were obtained by passive hemagglutination (5) and are expressed as reciprocals. –, Titers less than 1:40.

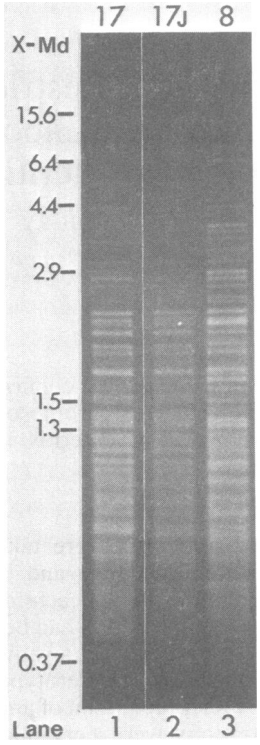

FIG. 1. Agarose gel electrophoresis of *Hin*dIII digests of *Campylobacter* chromosomal DNA. DNA was extracted by a modification of the procedure of McClure et al. (4), followed by digestion with the restriction endonuclease *Hin*dIII according to the specifications of the supplier (Boehringer Mannheim Corp., St. Laurent, P.Q.) with the exception that 20 to 30 U of *Hin*dIII were used per μg of DNA. *Hin*dIII digests were subjected to overnight electrophoresis at 30 V through 1.0% agarose and photographed by UV illumination after ethidium bromide staining. *Hin*dIII fragments of λ bacteriophage DNA of known molecular weight were electrophoresed in the same gel. Lane 1, Reference strain for *C. jejuni* serotype 17; lane 2, patient isolate (17J); lane 3, reference strain for *C. jejuni* serotype 8.

the absorbed antisera (Table 1). Antigenic preparations of the stool isolate reacted in antiserum 17 absorbed with serotype 8 but not in antiserum 8 after absorption with serotype 17. Since the titers of the antisera, particularly the absorbed antisera, were relatively low in comparison with titers of other *C. jejuni* antisera, another method of differentiating strains was employed.

In Fig. 1, the patterns of the bacterial restriction endonuclease digests of chromosomal DNA extracted from the isolate and the serotyping reference strains 8 and 17 may be compared. The procedures for extracting DNA and for electrophoresis, except for minor modifications, were the same as those of others described previously (1, 4). Clearly, the restriction pattern of serotype 17 reference strain (lane 1) and the isolate (lane 2) were not differentiable, but both were quite distinct from the pattern for the serotype 8 strain (lane 3).

Both serotyping and bacterial restriction endonuclease digest analysis linked the infection to one of the strains to which the worker had been exposed in the laboratory. The serotype strain 17, like other serotype reference strains of the serotyping scheme, had been passaged numerous times, and thus the results of this investigation indicate that such strains may retain their virulence even under long-term laboratory conditions of maintenance.

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