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Urease-Positive Thermophilic Campylobacter (*Campylobacter laridis* Variant) Isolated from an Appendix and from Human Feces

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Urease-positive thermophilic campylobacters were isolated for the first time from the feces of two adults with diarrheal disease and from the appendix of a child with appendicitis. They were identified as *Campylobacter laridis* by a hybridization dot blot assay. Urease testing should be included in the tests used for the identification of campylobacters at the species level, even for those strains which are not of gastric origin.

Campylobacter species other than *C. jejuni-C. coli* are increasingly isolated from human feces. *C. laridis* has been found in England (1), the United States (11, 12, 14), Canada (A. Borczyk, J. S. Thompson, D. Smith, H. Lior, and B. Devitt, *in* B. Kaijser, ed., *Campylobacter IV*, in press), and France (3). *C. hyointestinalis* has also been recovered from diarrheic patients (4, 5), as have "*C. upsaliensis*" (10, 13) and other strains which have not been well identified (10). The urease-positive thermophilic campylobacters (UPTC) have been described by Bolton et al. (2). The exact taxonomic position of UPTC is not known, and they have not been isolated from humans. We report here the first isolation of UPTC from human feces, and we describe their characterization as a variant of *C. laridis*.

The strains were isolated during a study of the incidence of *Campylobacter* infection, with samples arriving from a network of 25 hospital laboratories in France during the year 1986 to 1987. The strains were identified by using the following tests: growth at 25 and 42°C; growth in an anaerobic atmosphere in the presence of trimethyl amino oxide; growth with 1.5% NaCl; susceptibility to nalidixic acid and cephalothin; catalase, oxidase, and DNase activities; hippurate hydrolysis; nitrate reduction; urea hydrolysis; and H_2S production in triple sugar iron medium and izon-bisulfite-pyruvate (FBP) medium.

A hybridization procedure was also performed. Briefly, whole DNA was extracted from eight *Campylobacter* type strains, purified, and labeled with 2-acetylaminofluorene. The labeled DNAs were used as nonradioactive probes by using a previously described method (8) and a recently developed protocol (D. Chevrier, F. Mégraud, D. Larzul, and J. L. Guesdon, J. Infect. Dis., in press). The DNA of the strain to be identified was rapidly extracted, denatured in 0.1 M NaOH, and neutralized by adding 1.5 M NaH_2PO_4. Diluted DNA (400 μl) was spotted onto a nitrocellulose membrane by using a minifold apparatus (Schleicher & Schuell, Dassel, Federal Republic of Germany). After drying and baking (2 h at 80°C), the prehybridization and the hybridization were done by a standard procedure (7), with 250 ng of 2-acetylaminofluorene-labeled DNA per ml. After hybridization, the membrane was incubated successively with a monoclonal anti-2-acetylaminofluorene-labeled DNA antibody, alkaline phosphatase-labeled anti-mouse immunoglobulin G, and phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate [Boehringer GmbH, Mannheim, Federal Republic of Germany] and Nitro Blue Tetrazolium [Sigma Chemical Co., St. Louis, Mo.]). The stained membrane was dried, and the results were observed with the naked eye.

Two strains isolated by E. Bolton, Public Health Laboratory, Preston, England (C1295/85 and A19/82), were included in the study, as well as the *C. laridis* type strain, NCTC 11352. The three other strains were found among 788 *Campylobacter* strains studied. They were isolated in Paris, Annecy, and Bordeaux, France.

Case reports. The first patient was a 50-year-old man with a history of alcoholism, treated by vagotomy for a duodenal ulcer. He was hospitalized in February 1986 for acute watery diarrhea, abdominal pain, and fever (39°C) of 3 days' duration. Blood cultures were positive for *Streptococcus pneumoniae*, and radiologic signs of pneumopathy appeared 3 days later. Penicillin G was given for 10 days. At the same time, stool examination showed no leukocytes and a UPTC strain was isolated on Skirrow medium (Difco Laboratories, Detroit, Mich.) after 2 days of culture at 42°C. A negative result was recorded for *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica*.

The second patient was a 60-year-old woman suffering from a relapse of ovarian carcinoma, treated by surgery 4 years earlier and then by chemotherapy. A vesicorectal fistula appeared in August 1986. In December 1986, a coproculture was performed for diarrhea with pus and mucus and UPTC strains were isolated on Skirrow medium (Oxoid Ltd., Basingstoke, England) incubated at 37°C and on Campylosel (bioMérieux, Marcy l'Etoile, France) incubated at 42°C; both grew in a microaerobic atmosphere after 48 h. The stool specimen was negative for *Salmonella* spp., *Shigella* spp., *Y. enterocolitica*, *Aeromonas* spp., and *Clostridium difficile*. The episode was successfully treated with ampicillin and sisomycin.

The third patient was a 10-year-old boy hospitalized in August 1987 for acute appendicitis; the strain was isolated from a specimen of the inflamed appendix plated on a medium prepared by the method of Karmali (5a). Shortly after the operation, epigastric pain, as well as diarrhea and fever, appeared. No coproculture was performed, and a full recovery followed without antimicrobial treatment.

In the first case, the ingestion of a horse steak, refrigerated for a long time at 4°C, was suspected to be the cause of the disease, but no food sample was available for bacteriological confirmation.

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TABLE 1. Characteristics of UPTC strains and *C. laridis* type strain[a]

Strain	Test result[b]			
	Nal	H_2S TSI	Ure	NaCl
C. laridis NCTC 11352	R	−	−	W
A19/82	S	+	+	W
C1295/85	S	−	+	W
86-348	S	−	+	+
86-359	S	−	+	−
87-550	S	−	+	+

[a] All strains were positive for nitrates, catalase, and growth at 42°C; resistant to cephalothin; and negative for hippurate, DNase, and growth at 25°C.

[b] Nal, Nalidixic acid; H_2S TSI, production of H_2S in triple sugar iron medium; Ure, urease; NaCl, growth with 1.5% NaCl; +, positive; −, negative; R, resistant; S, susceptible; W, weak.

Characterization of strains. The characteristics of the three strains, as well as the strains from Bolton and the reference strain of *C. laridis*, are shown in Table 1. They were gram-negative curved rods and oxidase and catalase positive. They grew at 37 and 42°C but not at 25°C and were susceptible to nalidixic acid and resistant to cephalothin. They were nitrate reductase positive and hippurate negative; H_2S was not produced in FBP and triple sugar iron media, except for strain A19/82. A strong urease reaction was noted. Two of the strains grew well with 1.5% NaCl. They all grew anaerobically with trimethyl amino oxide.

By using semiquantitative hybridization, the three strains, as well as UPTC strains A19/82 and C1295/85, reacted strongly with the 2-acetylaminofluorene-labeled *C. laridis* probe; less than 10% hybridization was noted with *C. fetus*, *C. coli*, "*C. upsaliensis*," *C. sputorum*, *C. hyointestinalis*, and *C. pylori* probes, and only 10 to 30% was noted with the *C. jejuni* probe.

The importance of the urease test in the differentiation of *Campylobacter* species has been stressed since the discovery of *C. pylori*, isolated from the human stomach and duodenum (6). Other campylobacters, such as *C. nitrofigilis*, can hydrolyze urea but they have never been found in humans (9). Until now, UPTC strains were found only in the environment; 10 strains were isolated from river and sea water and from mussels and cockles (2). This is the first reported isolation from humans. The taxonomic position of UPTC strains has not been determined, but their sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profile (R. J. Owen, M. Costas, L. Sloss, and E. Bolton, *in* B. Kaijser, ed., *Campylobacter IV*, in press) and their fatty acid composition determined by gas liquid chromatography (M. J. Hudson and R. Wait, *in* B. Kaijser, ed., *Campylobacter IV*, in press) suggested that they were related to *C. laridis*. A whole-DNA hybridization technique permitted their identification as *C. laridis* without any doubt, and we classified them as *C. laridis* variants until a biotyping schema for *C. laridis* is available. These *C. laridis* variants, although rare, were more frequently isolated in France than the classical *C. laridis*. Only one strain of the latter was isolated during the same period. *C. laridis* strains have been found in sea gull feces (1); however, until now no mention has been made of UPTC strains in these birds. The urease test should be included in the group of tests developed for *Campylobacter* identification.

C. laridis was originally described as being nalidixic acid resistant, and this was the main characteristic used to differentiate it from *C. coli* in clinical laboratories. The existence of a group of nalidixic acid-susceptible *C. laridis* strains stresses the relativity of this characteristic. In the cases described here, we hypothesize that the strain had a role in the symptoms, but, in the first case as well as in many diarrheal episodes, no antibiotic was taken and the patient recovered spontaneously. More studies must be done to prove its role in diseases of the gastrointestinal tract.

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