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Isolation of "*Campylobacter hyointestinalis*" from a Human

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We isolated "*Campylobacter hyointestinalis*" from the rectal culture of a homosexual man with proctitis. Phenotypic characterization of the isolate was confirmed by DNA hybridization by using the taxonomic spot blot. "*C. hyointestinalis*" was previously isolated only from animals but should be considered in the identification of *Campylobacter* species isolated from humans.

Increasing awareness of the importance of *Campylobacter* species in human and animal infections has prompted workers in many laboratories to use appropriate microaerophilic conditions and selective isolation techniques when evaluating stool specimens. This has resulted in the increased recovery of *Campylobacter jejuni* and in the recognition of several new *Campylobacter* species, including *C. laridis* (1), "*C. cinaedi*" and "*C. fennelliae*" (2), and thermotolerant catalase-negative or weak *Campylobacter* species (9). "*Campylobacter hyointestinalis*," which was originally isolated from the intestines of swine with proliferative ileitis, is another new species (5). We isolated this organism from a homosexual man with proctitis and believe this to be the first report of "*C. hyointestinalis*" associated with human disease.

Case report. A 30-year-old homosexual man presented to the Harborview Medical Center Sexually Transmitted Diseases Clinic, Seattle, Wash., with a 6-day history of rectal discharge, nausea, anorexia, and periumbilical pain. He had no recent history of travel or unusual food consumption. Within the previous month he had had three casual sexual partners and had engaged in receptive anal intercourse without oral-anal contact. Physical examination was unremarkable except for bilateral, shotty inguinal lymphadenopathy. Anoscopic examination was notable for the presence of mucopus without friability. A sigmoidoscopy showed erythema and focal areas of mucosal bleeding up to 40 cm. Gram stain of a rectal swab revealed more than 15 leukocytes per oil immersion field (magnification, ×1,000) and no intracellular gram-negative diplococci. Rectal cultures were negative for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and herpes simplex virus; "*C. hyointestinalis*" was the only *Campylobacter* species isolated. Stool examination did not show leukocytes, erythrocytes, ova, or parasites, and stool cultures were negative for *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Vibrio* spp., and *Clostridium difficile*. The patient had a normal leukocyte count. The Venereal Disease Research Laboratory test was nonreactive. After he was treated empirically with 100 mg of doxycycline orally, twice daily for 1 week, the patient improved clinically. At follow-up visits 2, 9, and 38 days after the completion of therapy, rectal swab cultures were negative for "*C. hyointestinalis*" and no leukocytes were found with Gram-stained rectal swabs.

Microbiological studies. Brucella agar containing 10% sheep blood, vancomycin (10 mg/liter), polymyxin B (2,500 IU/liter), trimethoprim (5 mg/liter), and amphotericin B (2 mg/liter) was directly inoculated with a rectal swab collected during anoscopy. The culture was incubated at 35°C in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) containing a GasPak H_2+CO_2 generator envelope (BBL) without a catalyst and was first examined 96 h after inoculation. Colonies were yellow, raised, smooth, and nonswarming and did not have a distinctive odor. On Gram stain, the organisms were curved gram-negative rods but appeared thicker and less curved than *C. jejuni*. Darting motility was observed by dark-field microscopy. Further identification tests were performed as previously described (2). The isolate was oxidase and catalase positive, and it reduced nitrate, tolerated 1% glycine, produced H_2S in triple sugar iron agar (TSI), completely blackened a lead acetate strip suspended over TSI, and grew at 25 and 42°C. It did not produce acid from glucose or tolerate 2% NaCl. The organism was resistant to a nalidixic acid disk (30 μg) and susceptible to a cephalothin disk (30 μg). We determined the MIC of doxycycline for the isolate by agar dilution as previously described (3) but omitted sheep blood from the agar. The isolate was inhibited by 0.12 μg of doxycycline per ml.

Using the taxonomic spot blot procedure (13), we tested the isolate for DNA homology with "*C. hyointestinalis*." Radiolabeled DNA from the reference strain of "*C. hyoin-*

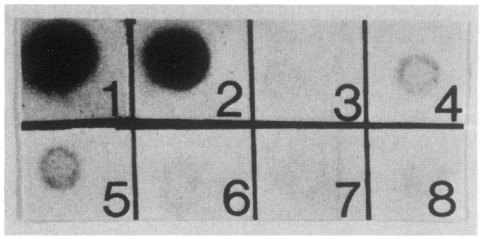

FIG. 1. Taxonomic spot blot with DNA isolated from "*C. hyointestinalis*" ATCC 35217 as the radiolabeled probe. The probe was allowed to hybridize with strains which had been treated and fixed to nitrocellulose. Panels: 1, strain 1287 (patient isolate); 2, "*C. hyointestinalis*" ATCC 35217; 3, *C. jejuni* C127 (CIP702); 4, *C. coli* C128 (CIP7080); 5, *C. fetus* subsp. *fetus* C16 (ATCC 27374); 6, *C. fetus* subsp. *venerealis* ATCC 19438; 7, "*C. fecalis*" C32 (144227A, B. D. Firehammer); 8, *C. laridis* NCTC 11352. The intense reactions of strains 1287 and ATCC 35217 indicated hybridization and thus homology with the probe DNA; weaker reactions were interpreted as low-level homology or background.

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TABLE 1. Presumptive differentiation of *Campylobacter* spp.[a]

Species	Susceptibility[b] to:		Test characteristic[c]							
	Nalidixic acid	Cephalothin	Catalase reaction	H_2S in TSI	Nitrate reduction	Hippurate hydrolysis	Odor	Yellow pigment	Growth at °C	
									25	42
"*C. hyointestinalis*"	R	S	+	+	+	−	−	V[d]	V[wd]	+
"*C. fecalis*"	R	S	+	+	+	−	−	−	−	+
C. fetus subsp. *fetus*	R	S	+	−	+	−	−	−	+	V[w]
C. laridis	R	R	+	−	+	−	−	−	−	+
C. jejuni	S	R	+	−	+	+	−	−	−	+
C. coli	S	R	+	−[e]	+	−	−	−	−	+
"*C. cinaedi*"	S	S	+	−	+	−	−	−	−	−
"*C. fennelliae*"	S	S	+	−	−	−	+	−	−	−
CNW[f]	S	ND[g]	−/+[w]	−	+	−	−	−	−	+
C. sputorum[h]	V	S	−	+	+	−	ND	V	V	V

[a] Data are from previous studies (1, 2, 4, 6, 8–10).
[b] R, Resistant; S, susceptible. Determined with 30-μg disks.
[c] +, Greater than 90% of strains were positive; −, less than 10% of strains were positive.
[d] V, 10 to 90% of strains were positive; w, weak reaction.
[e] *C. coli* produces a small band of H_2S if adequate water of syneresis is present (6, 8).
[f] CNW, Thermotolerant catalase-negative or weak *Campylobacter* sp.
[g] ND, Not determined.
[h] Includes *Campylobacter sputorum* subsp. *sputorum*, *Campylobacter sputorum* subsp. *bubulus*, and *Campylobacter sputorum* subsp. *mucosalis*.

testinalis" hybridized strongly with treated samples of the isolate and of the "*C. hyointestinalis*" reference strain but weakly or not at all with reference strains of *C. jejuni*, *Campylobacter coli*, *Campylobacter fetus* subsp. *fetus*, *Campylobacter fetus* subsp. *venerealis*, *C. laridis*, and "*Campylobacter fecalis*" (Fig. 1). We previously showed that the results of this semiquantitative test correlate well with quantitative whole-cell homologies of other *Campylobacter* species (13). The intense reaction of our isolate with the probe DNA from the "*C. hyointestinalis*" reference strain indicated substantial homology. This homology was easily distinguished from the weaker reactions of the "*C. hyointestinalis*" probe with the other *Campylobacter* species tested.

Discussion. Although very few phenotypic characteristics differentiate *Campylobacter* species, use of the tests shown in Table 1 would allow better characterization of unusual species in the routine microbiology laboratory and would indicate which isolates should be sent to reference laboratories for further identification. Temperature tolerance tests should be interpreted with caution because of variability in test parameters, such as incubator stability and inoculum size. Also, to consistently demonstrate H_2S production by "*C. hyointestinalis*" in TSI, the medium must be freshly prepared and incubated in an environment containing hydrogen (4).

With the use of this abbreviated scheme, *C. fetus* subsp. *fetus*, *Campylobacter sputorum*, and "*C. fecalis*" closely resemble "*C. hyointestinalis*," while other *Campylobacter* species are more easily distinguished. *C. fetus* subsp. *fetus* is differentiated from "*C. hyointestinalis*" by a lack of pigment, an inability to produce H_2S in TSI, and good growth at 25°C. In contrast to "*C. hyointestinalis*," *C. sputorum* strains are catalase negative. "*C. fecalis*" is distinguished from "*C. hyointestinalis*" by a lack of pigment and an inability to grow at 25°C. However, these two characteristics are only variably positive for "*C. hyointestinalis*," so separation of the two species is difficult unless further testing is performed. In a reference laboratory, the identification of "*C. hyointestinalis*" can be confirmed by taxonomic spot blot or by specialized tests with 3% NaCl, 1% glycine, and 0.04% triphenyltetrazolium chloride, as well as tests for anaerobic growth in 0.1% trimethylamine *N*-oxide hydrochloride, nitrite reduction, and hydrogenase activity (4).

Conditions favoring the isolation of *C. jejuni* from stool specimens are not optimal for the recovery of "*C. hyointestinalis*." Cephalothin (15 mg/liter) is commonly included in selective media. This concentration of cephalothin inhibited the growth of our isolate and the reference strain of "*C. hyointestinalis*" (data not shown). Also, although "*C. hyointestinalis*" tolerates 42°C, its growth is less abundant at that temperature than at 35°C (4).

The isolation of "*C. hyointestinalis*" from our patient in association with a proctitislike illness and the absence of other enteric and sexually transmitted pathogens suggests that the organism may have produced the illness of the patient. In addition, the organism disappeared and the symptoms and signs of infection resolved after treatment with an antibiotic to which the "*C. hyointestinalis*" strain was susceptible. Unfortunately, acute- and convalescent-phase sera from our patient were not available; thus, we could not confirm the etiologic role of "*C. hyointestinalis*" by serologic studies.

New species such as "*C. hyointestinalis*" are being isolated from the feces and blood of humans with increasing frequency (1, 2, 6, 7, 11, 12). Performance of the simple tests described here, in addition to the routinely performed Gram stain and oxidase procedures, is needed to more accurately identify these organisms so their role in disease can be better defined.

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