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Genotypic and Phenotypic Characterization of *Helicobacter cinaedi* and *Helicobacter fennelliae* Strains Isolated from Humans and Animals

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By DNA-DNA hybridization, we classified 26 human strains, 4 dog and cat strains, and 4 hamster strains putatively identified as *Helicobacter cinaedi* as well as 2 human strains and 2 animal strains of *Helicobacter fennelliae*. All but one human strain belonged to the same hybridization group as the type strain of *H. cinaedi*. The animal strains also appeared to belong to this hybridization group. Both human strains of *H. fennelliae* were shown to be *H. fennelliae* by DNA-DNA hybridization, but both animal strains were less than 15% related to the type strain. All strains were also characterized by plasmid profiles and ribotyping. Plasmids were found in 23% of the human strains, 100% of the hamster strains, and 33% of the dog and cat strains. Human strains were essentially identical by ribotyping, but were clearly differentiated from the hamster and dog and cat strains. Some strains may be difficult to culture on primary isolation; we found that our strains grew well on anaerobic CDC agar, brucella agar, and tryptic soy agar II. Our *H. cinaedi* and *H. fennelliae* strains differed from those previously described because some were resistant to cephalothin; some *H. cinaedi* strains were also resistant to nalidixic acid. All isolates were also characterized by antimicrobial susceptibility testing. We found that human strains of *H. cinaedi* were more resistant to clindamycin and erythromycin than were animal isolates; 19% of the human strains were resistant to ciprofloxacin. Therefore, we recommend that antimicrobial susceptibility results be obtained before initiating therapy for *H. cinaedi* and *H. fennelliae* infections.

In a study of campylobacters associated with diarrhea in homosexual men, several groups of fastidious *Campylobacter*-like organisms were isolated (19). Subsequent hybridization studies subdivided these organisms into four DNA relatedness groups or genomospecies: *Campylobacter cinaedi* (CLO-1A), CLO-1B, *Campylobacter fennelliae* (CLO-2), and CLO-3 (6, 25). CLO-1B was not designated as a separate species, because it could not be separated phenotypically from *C. cinaedi*. Other investigators subsequently proposed that *C. cinaedi* and *C. fennelliae* be transferred to the genus *Helicobacter* on the basis of DNA-rRNA hybridization data (27).

Following the original hybridization studies, investigators have designated strains as *Helicobacter cinaedi* on the basis of various nongenotypic techniques, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8, 26), immunoblotting with *H. cinaedi*-specific antiserum (8), and immunotyping (26). Another group of investigators used nonquantitative hybridization techniques to classify additional strains as *H. cinaedi* (10, 22).

The objectives of the present study were as follows. First, we classified strains putatively identified as *H. cinaedi* and *Helicobacter fennelliae* by quantitative DNA-DNA hybridization. Second, we characterized all strains by standardized phenotypic and antimicrobial susceptibility testing, because atypical phenotypic characteristics that may cause difficulty in identification (e.g., resistance to cephalothin and nalidixic acid) had been noted for some strains. Finally, we investigated the ability of the *H. cinaedi* and *H. fennelliae* strains to grow on media typically available in a clinical microbiology laboratory.

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MATERIALS AND METHODS

Strains. Strains were included in this study if they were shown by cellular fatty acid analysis to have a profile similar to that of *H. cinaedi* or *H. fennelliae* (15), without regard to phenotypic characteristics (Table 1). Strains were isolated between 1982 and 1991 and were forwarded to the Centers for Disease Control and Prevention (CDC) for identification. Strains received at the CDC were frozen as soon as possible at −70°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 20% glycerol. Strains from dogs and cats included in this study were originally isolated as part of a study comparing selective media for the isolation of *Campylobacter* (16). Isolates from hamsters were kindly provided by Connie Gebhart (University of Minnesota, St. Paul) (8).

DNA-DNA hybridization. To obtain sufficient amounts of DNA, organisms were grown on 25 to 50 plates (150 by 15 mm) containing diagnostic sensitivity test (DST) agar (Oxoid, Ogdensburg, N.Y.) with 10% horse blood (DSTHB) and incubated for a minimum of 48 h in an anaerobic incubator containing approximately 5% oxygen, 7.5% carbon dioxide, 7.5% hydrogen, and 80% nitrogen. Cells were harvested and DNA was purified as previously described (2), with one notable exception: cells lysed extremely quickly, and it was necessary to add phenol heated to 60°C immediately after adding sodium dodecyl sulfate (decreased to 0.125% from 0.5%). The lysing solution was prepared without pronase. DNAs from strains were labeled with $^{32}PO_4$ with commercial nick translation reagents (Promega, Inc., Madison, Wis.) and tested for reassociation with unlabeled homologous and heterologous reaction mixtures as described by Brenner et al. (2). Temperatures of 55 and 70°C were used for optimal and stringent reassociations, respectively. Percent divergence, defined as the percentage of unpaired bases within related DNA sequences, was determined by assuming that each 1% decrease in thermal stability is caused by approximately 1% unpaired bases (2). Percent divergence was calculated to the nearest 0.5%. Results given for relatedness are those obtained under optimal conditions, unless indicated otherwise.

Ribotyping. All strains were characterized by ribotype following restriction with *Pvu*II and hybridization with rRNA labeled with digoxigenin as previously described (12, 13, 28). We have not yet been able to identify additional restriction enzymes that restrict *H. cinaedi* DNA in order to further discriminate between

TABLE 1. Strains used in this study

Type of organism	Strain(s) or designation(s)	Catalog no.
Human strains putatively identified as *H. cinaedi* on the basis of cellular fatty acid analysis[a]	D418 (ATCC 35683), D413, D1576, D1659, D1933, D2084, D2094, D2204, D2205, D2315, D2370, D2394, D2449, D2629, D2636, D2724, D2728, D2794, D2820, D2910, D3828, D3885, D3913, D3914, D3920, E8774, F2682, D3929, D3930, D3965, D3968, D4124, D2604	
Animal strains putatively identified as *H. cinaedi* on the basis of cellular fatty acid analysis	Sk425 (cat), Sk374 (dog), Sk383 (dog), Sk417 (dog), D2740 (hamster), D2741 (hamster), D2742 (hamster), D2743 (hamster), D2744 (hamster), D2745 (hamster), D2207 (hamster)	
Human strains putatively identified as *H. fennelliae* on the basis of cellular fatty acid analysis	D420 (ATCC 35684), D2734, D2609	
Animal strains putatively identified as *H. fennelliae* on the basis of cellular fatty acid analysis	D3858 (macaque), Copper (dog)	
CLO1B strain	D419	
Type and reference strains[b]	*Campylobacter jejuni* (D133)	ATCC 33560
	Campylobacter coli (D145)	ATCC 33559
	Campylobacter fetus subsp. *fetus* (D373)	ATCC 27374
	Campylobacter fetus subsp. *venerealis* (D374)	
	Campylobacter lari (D375)	NCTC 11352
	Campylobacter fecalis (D1169)	NCTC 11415
	Campylobacter mucosalis (D1179)	ATCC 43265
	Campylobacter sputorum subsp. *bubulus* (D1248)	ATCC 33562
	Campylobacter upsaliensis (D1914)	NCTC 11541
	Campylobacter hyointestinalis (D1932)	ATCC 35217
	Campylobacter concisus (D2082)	ATCC 33237
	Campylobacter rectus (D2083)	ATCC 33238
	Campylobacter jejuni subsp. *doylei* (D2295)	
	Helicobacter pylori (D2371)	ATCC 43504
	Arcobacter cryaerophilus 1B (D2610)	ATCC 49615
	Arcobacter butzleri (D2686)	ATCC 49616
	Arcobacter nitrofigilis (D2711)	ATCC 33309
	Campylobacter curvus (D2712)	ATCC 35224
	Wolinella succinogenes (D2713)	ATCC 29543
	Arcobacter cryaerophilus 1A (D2792)	ATCC 43158
	Helicobacter mustelae (D2973)	ATCC 43772
	962 (hamster)	

[a] All of these strains—with the exception of D418, D2084, D3920, E8774, D3929, D3930, D3965, D3968, D4124, and D2604—were associated with bacteremia and are described in another publication (14). Strains D3929, D3930, D3965, D3968, and D4124 were not characterized by DNA-DNA hybridization but are included in phenotypic and antimicrobial characterization because they have been shown by ribotyping to be identical to the type strain of *H. cinaedi*. Strain D2604 was also putatively identified as *Campylobacter fetus* subsp. *fetus.*

[b] Type and reference strains were obtained from the sources listed in a previous publication (11).

isolates identical in *Pvu*II digests. Isolates were initially classified as *H. cinaedi* if they displayed a pattern essentially identical to that of the type strain.

Plasmid analysis. All strains were examined for the presence of plasmids by the modified Portnoy technique (18) described by Tenover et al. (23). In addition, we treated plasmid DNA with RNase (10 mg/ml) after extraction. Plasmids were visualized following electrophoresis on 0.7% vertical agarose gels run in Tris-borate-EDTA buffer.

Phenotypic tests. All strains were characterized by the methods described by Barrett et al. (1), except that growth on MacConkey agar was determined by the plate method (11). Tests performed included growth under aerobic and anaerobic conditions; growth at 25, 35, and 42°C; growth in the presence of 1.5% NaCl, 3.5% NaCl, and 1% glycine and on MacConkey agar; production of hydrogen sulfide in triple sugar-iron agar and on lead acetate strips and of oxidase and catalase; reduction of nitrate, hippurate, and indoxyl acetate (17); susceptibility to nalidixic acid and cephalothin disks (30 μg); and ability to oxidize or ferment glucose. In addition, because previous experience suggested that many clinical laboratories have difficulty culturing these organisms on solid media (14), we examined the abilities of these isolates to grow on media typically available in a hospital microbiology laboratory: brucella agar with 5% sheep blood, hemin, and vitamin K (catalog no. 01-254; Remel, Lenexa, Kans.); Anaerobic CDC agar (catalog no. 01-036; Remel); chocolate agar (catalog no. 01-302; Remel); tryptic soy agar II with 5% sheep blood (catalog no. 21239/21261; BBL Microbiology Systems, Cockeysville, Md.); and supplemented brain heart infusion agar (catalog no. 01-1128-03; Carr-Scarborough Microbiologicals, Decatur, Ga.). All plates were inoculated with a swab saturated with a suspension of organism prepared in tryptic soy broth (BBL) to a density equivalent to a 1.0 McFarland standard.

Finally, we investigated the three tests proposed by Burnens and Nicolet (3), arylsulfatase activity, pyrazinamidase activity, and susceptibility to polymyxin B, for better discrimination of *Campylobacter* spp. and related organisms. Tests were initially performed as described previously. Arylsulfatase and pyrazinamidase tests were repeated for 20 isolates, with DST agar substituted as the base; the base medium had no apparent effect on the results of the arylsulfatase and pyrazinamidase tests. To test for polymyxin resistance, we also compared suspensions equivalent to both 0.5 and 1.0 McFarland standards for inoculating the medium (since tests for nalidixic acid and cephalothin resistance are determined with a 1.0-McFarland standard suspension). Zone diameters were essentially the same for both inoculum concentrations tested for the 43 *H. cinaedi* and *H. fennelliae* isolates tested and the 10 representative *Arcobacter* and *Campylobacter* isolates tested.

Antimicrobial susceptibility testing. Susceptibility testing was performed by the agar dilution method. Antimicrobial agents were obtained directly from the appropriate pharmaceutical manufacturer. Initially, plates containing the appropriate dilutions of antimicrobial agents tested were prepared with brucella-sheep blood agar (7); trimethoprim and trimethoprim-sulfamethoxazole were prepared with DSTHB agar (7). However, because we noted significantly improved growth on DSTHB, repeat testing was performed with this agar. Most results reported

TABLE 2. Relatedness of *H. cinaedi* type strain D418 and *H. fennelliae* type strain D420 to strains putatively identified as *H. cinaedi* and *H. fennelliae*[a]

Strain	Result (%) for strain:													
	D418			D420			Sk417			Sk425			D2741	
	Optimal reassociation at 55°C	Divergence	Stringent reassociation at 70°C	Optimal reassociation at 55°C	Divergence	Stringent reassociation at 70°C	Optimal reassociation at 55°C	Divergence	Stringent reassociation at 70°C	Optimal reassociation at 55°C	Divergence	Stringent reassociation at 70°C	Optimal reassociation at 55°C	Divergence
D418	100	0.0	100	26			66	7.0	42	38	2.5	78	89	0.0
D3914	93	0.0	96											
D3828	93	0.5	89	23			62	7.0	42	53	3.5	76		
D3920	100	0.5	98											
D413	92	0.5	89	25			57	7.5	39	74	4.0	76		
D2910	77	2.5	59											
E8774	81	3.5	73				50	8.0	24	70	7.5	51	71	1.5
D2629	88													
D2794	89													
D2205	88													
D2394	94													
D2636	87	0.5	85											
D2724	84													
D2449	80	0.0	82											
D2315	95	0.5	90											
D1576	100	1.0	91											
D2094	99	3.0												
D3913	84													
D2820	94													
D2204	85													
D3885	98													
D1659	81													
D2728	84													
F2682	84													
D1933	89													
D2370	90													
Sk425	69	3.0	55				64	4.5	40					
Sk374							61	4.5						
Sk383	71	2.0	63				58	5.5	42	83	2.0	88		
Sk417	69	3.5	63	7			100	0.0	100	68	1.5	79	79	0.0
D2741	61	8.5	30				50	8.0	29	53	5.5		100	0.0
D2743	58	9.0	26				41	11.0	25					
D2744	71	3.5	58	8			60	6.5	43	84	2.5	89	100	0.0
D2207													100	0.0
D2084	37	10.5	14	0			35						33	
D2740	16													
D2742	11													
D420	45	4.0	45	100	0.0	100				28	9.5	25	31	
D2734				100	0.5	88								
D2609				100	2.0	100								
D3858				13	9.5	24								
Copper	19	9.0	11	3			63	9.0	40	2	10.0	6	15	
D419	60	6.5	33	12			44	12.0	19	75	8.5	54	69	3.0
962	9			2			9			0	9.5	7	4	
D2604	4			1			13			10	2.5	5	4	
D2371	4			4			11			2	7.5	2	1	
D2973			1	4			3			1	4.5	2	1	
D133	6			24			5						1	
D145	5			21			12						3	
D1169	4			4			6						2	
D1179	5			10			3						1	
D1248	4			7			6						1	
D1914	9			15			10						3	
D1932	13						6						2	
D2082	5			3			11						0	
D2083	2			2									0	
D2295	19			19			7						11	
D2712	3			19			6						0	
D373	4			2			10			1	17.5	3	2	
D374	5			2			10			7	7.5	1	0	
D375	5			15			13						1	
D2711	3			12			3						0	
D2610	3			5			5						0	
D2792	2			32			8						0	
D2686	3			3	12.5	19							5	
D2713	5			0			9						1	

[a] Values for 55 and 70°C are binding ratios relative to the reference strain. Divergence is defined as the percentage of unpaired bases within related DNA sequences.

(except those for amoxicillin-clavulanic acid, cefoxitin, nalidixic acid, oxacillin, and vancomycin) are from DSTHB. All antimicrobial agents were tested in a series of twofold dilutions from 0.06 to 128.0 μg/ml, except for ciprofloxacin, which was tested from 0.006 to 8 μg/ml, and trimethoprim-sulfamethoxazole, which was tested from 1.19/0.06 to 76/4 μg/ml. The final inoculum was approximately 10^4 CFU per spot. Plates without antimicrobial agents were inoculated to ensure growth of each strain under the same conditions. All plates were incubated under microaerobic conditions for 48 h. MICs were defined as the minimum concentration of the antimicrobial agent which completely inhibited growth of the strain, except for those of trimethoprim and trimethoprim-sulfamethoxazole, for which 80% inhibition of growth was determined as the endpoint.

RESULTS

Results obtained from DNA-DNA hybridization studies are shown in Table 2. With the exception of strain D2084, all human strains belonged to the same hybridization group as the type strain of *H. cinaedi* and were 77 to 100% related to the type strain. All but two of the strains isolated from animals also were in, or close to, this hybridization group on the basis of direct relatedness to the *H. cinaedi* type strain or on the basis of relatedness to another strain that was related to *H. cinaedi* at the species level. In general, relatedness of animal strains to the *H. cinaedi* type strain was significantly lower than that seen with human strains. The two hamster isolates originally thought to phenotypically resemble *H. cinaedi* (D2740 and D2742) belonged to a new species of *Campylobacter* that is described elsewhere (9) and were not studied further. The type strain of *H. cinaedi* was less than 20% related to any currently recognized *Campylobacter* or *Arcobacter* species, *Helicobacter pylori*, and *Helicobacter mustelae.*

Two human strains belonged to the same hybridization group as the type strain of *H. fennelliae* and were essentially 100% related to that strain. The type strain of *H. fennelliae* was less than 25% related to *Campylobacter* species, 12 to 32% related to *Arcobacter* species, and <5% related to *Wolinella succinogenes*, *H. pylori*, and *H. mustelae*. The *H. fennelliae*-like strains (D3858 and Copper) isolated from animals did not belong to the same hybridization group as the type strain. Further hybridization studies to analyze the relationship between these two strains and to other *Arcobacter*, *Campylobacter*, or *Helicobacter* strains were not done.

All strains were also characterized by ribotyping. Strains of *H. cinaedi* from different host sources were clearly distinguishable: human *H. cinaedi* strains exhibited a ribotype pattern essentially identical to that of the type strain; hamster isolates differed from both human isolates and from dog and cat isolates. Results typical of *H. cinaedi* strains from humans, dogs and cats, and hamsters are shown in Fig. 1; also shown are results for D2084. Figure 1 also includes the human *H. fennelliae* isolates and the two *H. fennelliae*-like isolates from animals. Diversity was noted for the *H. fennelliae* isolates; these strains were also clearly differentiated from *H. fennelliae*-like isolates obtained from animals.

The results of phenotypic characterization are shown in Table 3. The human strains of *H. cinaedi* were indistinguishable phenotypically from the animal strains. D2084 differed from *H. cinaedi* only by its lack of growth at 42°C. Sk417 was differentiated from the other dog and cat strains by the fact that it was H_2S negative, resistant to cephalothin, and nitrate negative. Twenty-nine percent of the *H. cinaedi* strains were resistant to nalidixic acid, and 81% of the *H. cinaedi* strains were resistant to cephalothin; 33% of the *H. fennelliae* strains were resistant to cephalothin. The two *H. fennelliae*-like strains isolated from animals differed from human *H. fennelliae* strains in that they did not grow in the presence of glycine and did not have arylsulfatase activity. *H. cinaedi* and *H. fennelliae* strains included in this study grew best on anaerobic CDC agar. Brucella agar and tryptic soy agar II containing 5% sheep blood generally supported the growth of *H. cinaedi* and *H. fennelliae* isolates and were significantly better than chocolate or supplemented brain heart infusion agars.

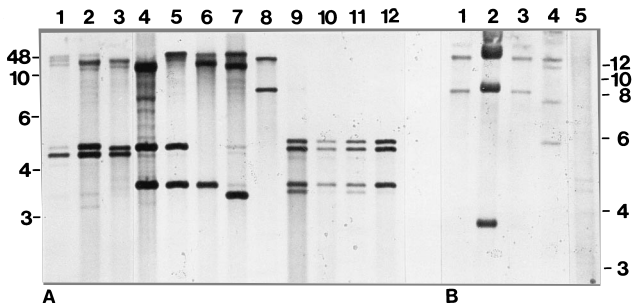

FIG. 1. (A) Ribotyping patterns from representative *H. cinaedi* isolates from humans and animals. Lanes 1 and 2, two representative human isolates (D3914 and F2682, respectively); lane 3, type strain (D418); Lane 4, human strain that did not belong to the *H. cinaedi* hybridization group (D2084); lanes 5 to 8, dog and cat isolates (Sk374, Sk383, Sk425, and Sk417, respectively); lanes 9 to 12, hamster isolates (D2741, D2743, D2744, and D2745, respectively). (B) Ribotyping patterns from *H. fennelliae* and *H. fennelliae*-like strains. Lane 1, type strain of *H. fennelliae*; lanes 2 and 3, two human strains (D2734 and D2609, respectively); lane 4, dog strain (Copper); lane 5, macaque strain (D3858).

After noting that many of our *H. cinaedi* and *H. fennelliae* strains were resistant to cephalothin and/or nalidixic acid, we further characterized their antimicrobial susceptibilities (Table 4). The MICs for strains D2084, Sk417, Copper, and D3858 are shown separately because they do not clearly belong to the other hybridization groups. Significant differences between human and animal isolates of *H. cinaedi* were found, with increased levels of resistance to erythromycin, clindamycin, and kanamycin among human strains. Cross-resistance between erythromycin and clindamycin was always noted for human strains. In addition, resistance to nalidixic acid correlated with resistance to ciprofloxacin for 6 of 10 human strains but not for animal strains. Several antimicrobial agents commonly found in selective media were included in this analysis (cefazolin, cephalothin, trimethoprim, and vancomycin). All strains were resistant to trimethoprim and vancomycin, with the exception of D3858, which was susceptible to trimethoprim. Many of our strains of *H. cinaedi* and *H. fennelliae*, as well as animal isolates included in this study, were inhibited by concentrations of cephalothin and cefazolin typically included in selective media.

Following recognition that antimicrobial resistance patterns typically indicative of plasmids were present in our strains, all strains were examined by a modified Portnoy method. Plasmids were found in 7 of 31 (23%) human strains, compared with 6 of 6 hamster *H. cinaedi* isolates and 1 of 3 dog and cat *H. cinaedi* isolates. Results are shown in Table 5. The smaller plasmids (1.8 and 1.4 MDa) appeared to be correlated with tetracycline and chloramphenicol resistance. The 30-MDa plasmid was detected in 13 strains and was difficult to correlate with antimicrobial-agent resistance.

DISCUSSION

Our results indicate that most of our isolates of *H. cinaedi* form a single group, both phenotypically and by DNA-DNA hybridization. There may be subgroups within *H. cinaedi* that correlate with the host source; however, because of the extreme difficulty we encountered in obtaining adequate amounts of DNA, we were unable to obtain sufficient data to delineate these groups. Subgroups are presently distinguishable by ribotyp-

TABLE 3. Phenotypic characteristics of *H. cinaedi* and *H. fennelliae* strains from humans and animals

Test	% Positive results with:						
	Human *H. cinaedi* isolates (n = 31)[a]	D2084 (n = 1)	Hamster isolates (n = 5)	Dog and cat isolates (n = 3)	Sk417 (n = 1)	Human *H. fennelliae* isolates (n = 3)	Animal *H. fennelliae*-like isolates (n = 2)
Growth under anaerobic conditions[b]	26[c]	0	0	33[c]	0	33	50[c]
Growth at 42°C[d]	100[e]	0	100	100	100	100	50
Growth on or in the presence of[f]:							
Glycine (1.0%)	87	100	100	100	100	100	0
NaCl (3.5%)	10	0	0	0	0	0	0
Production or presence of[g]:							
Catalase	79	Trace	100	100	100	100	50
Motility	100	100	100	100	100	100	0
H_2S-triple sugar-iron	0[h]	0	0	0[h]	0	0	0
H_2S-lead acetate	42	Trace	80	100	0	100	50
Resistance to:							
Nalidixic acid	29	0	80	0	0	0	0
Cephalothin	81	100	100	0	100	33	0
Polymyxin B	97[i]	100	100	100[i]	100	100	100[i]
Reduction or hydrolysis of[j]:							
Nitrate	100	100	100	100	0	0	0
Indoxyl acetate	6	0	0	0	0	100	100
Arylsulfatase	0[i]	0	0	0[i]	0	100	0[i]
Pyrazinamidase	0[i]	0	0	0[i]	0	0	0[i]

[a] Includes five strains not tested by DNA-DNA hybridization but shown by ribotyping to be identical.
[b] No strains exhibited growth under aerobic conditions.
[c] Demonstrated trace growth under these conditions.
[d] No strains exhibited growth at 25°C, whereas all strains exhibited growth at 35°C.
[e] Growth was apparent but considerably less than that at 35°C.
[f] No strains exhibited growth on MacConkey agar or in the presence of NaCl at 1.5%. All strains lacked the ability to oxidize or ferment glucose.
[g] All strains produced oxidase.
[h] Many strains did not grow on triple sugar-iron medium; negative results are presented for strains that did exhibit growth.
[i] Four human strains (D413, D2794, D2820, and D3913), one cat isolate (Sk383), and the macaque isolate (D3858) were not available at the time of determination of polymyxin B susceptibilities and production of arylsulfatase and pyrazinamidase.
[j] No strains hydrolyzed nitrite or hippurate.

ing; the human strains form a homogeneous group, but the hamster isolates most closely resemble *Campylobacter concisus* by ribotyping. The ribotyping patterns of the dog and cat isolates do not resemble those of either the hamster or the human isolates.

We found only two strains of *H. fennelliae* in the CDC collection. The first strain (D2734) was from a woman with bacteremia, and the second strain (D2609) was from the stool of an asymptomatic child. These strains demonstrated similar but different ribotyping patterns from those of the type strain and from each other and had no bands in common with either animal strain. Although the animal strains phenotypically resembled *H. fennelliae* and had been characterized as *H. fennelliae* by gas-liquid chromatographic analysis of cellular fatty acids, they were not related to *H. fennelliae* at the species level. However, we were unable to further analyze their relationship to each other or to other type strains by DNA hybridization because we did not have sufficient DNA. Following completion of our study, Burnens et al. described several isolates from dogs that resembled *H. fennelliae* (4). It is possible that these strains may be related to one or both of our strains; however, there are phenotypic differences, and further work would be necessary to delineate any relationships between the two groups of strains.

Few significant differences between the phenotypic characterizations of our strains and those of the original isolates described by Fennell et al. (6) were seen. The most important difference was that many of our strains were resistant to either nalidixic acid or cephalothin. *H. cinaedi* strains resistant to both nalidixic acid and cephalothin may be difficult to differentiate from *Campylobacter lari*; however, in contrast to *C. lari* strains, *H. cinaedi* strains will not grow on MacConkey agar or in the presence of 1.5% NaCl. Resistance to cephalothin but not to nalidixic acid was found in 33% of the *H. fennelliae* strains. *H. fennelliae* strains can be differentiated from other catalase-positive, indoxyl acetate-positive strains by their inabilities to hydrolyze hippurate or nitrate or to grow at 25°C. More of our *H. cinaedi* and *H. fennelliae* strains grew under anaerobic conditions and at 42°C than previously reported; however, this may represent laboratory adaptation of strains or a preference of the strains for the controlled microaerobic atmosphere we used for incubation at 42°C. We noted hydrogen sulfide production on lead acetate paper for 42% of our *H. cinaedi* isolates, compared with 100% for the strains described by Fennell et al. (6). Our results agreed with those of Burnens and Nicolet for the three supplementary diagnostic tests (3): essentially all strains were resistant to polymyxin B, *H. fennelliae* strains were arylsulfatase positive, and all strains were negative for pyrazinamidase. We felt that the most useful test was that for sensitivity to polymyxin B but modified the test so that polymyxin B was inoculated from a suspension equivalent

TABLE 4. Antimicrobial susceptibilities of *H. cinaedi* and *H. fennelliae* strains from human and animals[a]

Antimicrobial agent	Value(s) (μg/ml) for:												
	Human *H. cinaedi* isolates					Dog and cat *H. cinaedi*-like strains			Hamster *H. cinaedi*-like strains			MIC for Copper	MIC for D3858
	Range of MIC	Mode of MIC	MIC_{50}	MIC_{90}	MIC for D2084	Range of MIC	MIC_{50}	MIC for Sk417	Range of MIC	Mode(s) of MIC	MIC_{50}		
Aminoglycosides													
Gentamicin	0.12–16.0	0.25	0.25	0.5	0.25	0.25–1.0	0.25	0.5	0.12–0.5	0.25	0.25	0.5	0.12
Kanamycin	≤1.0–32.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	16.0	≤1.0
Cephalosporins													
Cefazolin	≤1.0–64.0	4.0	4.0	16.0	32.0	2.0–8.0	8.0	8.0	≤1.0–>64.0	≤1.0	4.0	8.0	32.0
Cephalothin	16.0–>128.0	64.0	64.0	128.0	>128.0	32.0–64.0	64.0	32.0	1.0–128.0		4.0	32.0	4.0
Cefoxitin	16.0–128.0	>128.0	>128.0	>128.0		>128.0	>128.0	4.0	16.0–>128.0	>128.0	128.0	8.0	
Ceftriaxone	0.25–>8.0	8.0	8.0	8.0	8.0	4.0	4.0	1.0	8.0–>8.0	8.0	8.0	>8.0	≤0.12
Chloramphenicol	0.25–4.0	1.0	1.0	2.0	2.0	1.0–4.0	2.0	4.0	1.0–4.0	1.0, 2.0	2.0	>8.0	0.5
Clindamycin	≤0.06–>128.0	8.0	8.0	>128.0	>128.0	0.12–0.5	0.25	0.5	0.25	0.25	0.25	>128.0	≤0.06
Erythromycin	≤0.06–>128.0	>128.0	128.0	>128.0	128.0	≤0.06–0.5	0.12	0.12	≤0.06–0.12	≤0.06	≤0.06	>128.0	≤0.06
Penicillins													
Ampicillin	1.0–32.0	8.0	8.0	16.0	32.0	8.0	8.0	2.0	≤0.5–32.0		8.0	2.0	2.0
Amoxicillin-clavulanic acid	0.25–8.0	2.0	2.0	4.0		4.0–8.0	4.0	1.0	1.0–16.0	1.0, 4.0	4.0	2.0	
Oxacillin	16.0–128.0	32.0	32.0	64.0		32.0–64.0	32.0	16.0	8.0–>128.0	8.0, 32.0	32.0	64.0	
Quinolones													
Nalidixic acid	8.0–128.0	16.0	16.0	64.0		8.0–16.0	8.0	8.0	8.0–16.0	8.0	8.0	16.0	
Ciprofloxacin	0.12–>8.0	0.25	0.25	>8.0	0.25	0.12–1.0	0.12	0.12	0.12–0.5	0.25, 0.5	0.5	4.0	≤0.06
Trimethoprim	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	≤128.0
Sulfamethoxazole-trimethoprim[b]	9.5/0.5–>76/4	38/2	38/2	38/2	≤38/2	≤1.1/0.06–76/4	38/2	>128.0	9.5/0.5–76/4	76/4	76/4	38/2	
Tetracyclines													
Doxycycline	≤0.06–2.0	0.25	0.25	1.0	0.25	≤0.06–0.12	0.12	0.12	≤0.06–0.5	0.5	0.5	4.0	≤0.06
Tetracycline	0.25–8.0	0.5	1.0	8.0	4.0	0.25–0.5	0.25	0.25	0.5–4.0	4.0	1.0	16.0	≤0.06
Vancomycin	>128.0	>128.0	>128.0	>128.0		>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0	>128.0

[a] MIC_{50} and MIC_{90}, MICs at which 50 and 90%, respectively, of the isolates are inhibited.
[b] MIC of trimethoprim/MIC of sulfamethoxazole.

TABLE 5. Plasmid analysis of *H. cinaedi* and *H. fennelliae* strains

Strain	Genetic classification	Molecular mass(es) of plasmid(s) (MDa)
D2449	Human *H. cinaedi* isolate	30
D2910	Human *H. cinaedi* isolate	1.8
D3828	Human *H. cinaedi* isolate	30
D3920	Environmental *H. cinaedi* isolate associated with human illness	30
D3929	Human *H. cinaedi* isolate	30
D3930	Human *H. cinaedi* isolate	30
D3968	Human *H. cinaedi* isolate	30
D2084	Human *H. cinaedi*-like isolate genetically distinct from those in all other groups	30
D2207	Hamster *H. cinaedi* isolate	30
D2741	Hamster *H. cinaedi* isolate	30
D2743	Hamster *H. cinaedi* isolate	30
D2744	Hamster *H. cinaedi* isolate	30
D2745	Hamster *H. cinaedi* isolate	30
Sk417 (flat colony)	Dog *H. cinaedi* isolate	1.5
Sk417 (green colony)	Dog *H. cinaedi* isolate	30
Copper	Dog *H. fennelliae*-like isolate	32, 1.4, 1.2

to a 1.0 McFarland standard; the other two tests require media that are not commercially available, and identification could be confirmed by other means. The phenotypic characteristics of the two *H. fennelliae*-like strains isolated from animals differ from those described by Burnens et al. (4), because our strains hydrolyzed indoxyl acetate.

Our antimicrobial susceptibility results differ from those previously reported (7) for *H. cinaedi* in that our human strains were more resistant to clindamycin, erythromycin, and nalidixic acid. In addition, we found 6 of 31 (19%) strains to be resistant to ciprofloxacin. Because the majority of our human strains resistant to nalidixic acid were also resistant to ciprofloxacin, it may be reasonable to suggest to clinicians that patients with *H. cinaedi* strains resistant to nalidixic acid should not be treated with ciprofloxacin unless MICs of ciprofloxacin are available. This is in contrast to suggestions by others that this antimicrobial agent is effective in treating patients infected with *H. cinaedi* (5, 20).

We detected plasmids in 35% of our strains; this is essentially the same as results previously reported for *Campylobacter jejuni* and *Campylobacter coli* (24). Our results suggest that plasmid analysis may be of some epidemiologic utility and that the potential of ribotyping for epidemiologic purposes is minimal, because *Pvu*II ribotype patterns from human strains are essentially identical.

The fact that most of our strains were isolated from blood is probably explained by three difficulties one would likely encounter when attempting to isolate *H. cinaedi* and *H. fennelliae*, particularly from stool samples and other nonsterile sources. Most importantly, *H. cinaedi* and *H. fennelliae* do not grow well at 42°C. Furthermore, our antimicrobial susceptibility studies indicate that many of our strains would have been inhibited by some of the antibiotics (e.g., cephalosporins) typically contained in selective media. Finally, our observations indicate that the presence of hydrogen is necessary for optimal growth and may be most important during primary isolation (data not shown).

Both the present study and a previous investigation indicate that *H. cinaedi* and *H. fennelliae* organisms may be susceptible to antimicrobial agents contained in selective media (i.e., cephalothin and cefazolin). With the exception of the macaque strain (D3858), none of the *H. cinaedi* or *H. fennelliae* strains were inhibited by trimethoprim or vancomycin. Although we did not determine the MICs of polymyxin B, we did note resistance by the disk method. One alternative to selective media may be the use of filtration (21) to selectively isolate *H. cinaedi* and *H. fennelliae* organisms from contaminated specimens. For the isolation of *H. cinaedi* and *H. fennelliae* from normally sterile sources (e.g., blood cultures), our experience suggests that *H. cinaedi* and *H. fennelliae* are recovered by plating the blood culture broth onto a nonselective medium such as anaerobic CDC agar or agar containing 5% sheep blood (brucella or Mueller-Hinton agar or tryptic soy agar II).

None of the commercial systems available in the United States provide adequate amounts of hydrogen for optimal growth of *H. cinaedi* and *H. fennelliae*. The system used by Fennell et al. (6) provided an atmosphere containing approximately 16 to 18% hydrogen, but this mixture is highly flammable and is not recommended by the manufacturer. The atmospheric mixture typically used at the CDC may be difficult for other investigators in the United States to obtain because >6% hydrogen is considered flammable. In addition, the current manufacturer of the anaerobic incubator (catalog no. 52201-462; VWR Scientific, South Plainsfield, N.J.) no longer recommends that a hydrogen concentration greater than 5% be used in the incubator. Therefore, an alternative gas mixture of 6% hydrogen, 10% carbon dioxide, and the balance nitrogen may be used. This alternative provides a final gas concentration of 5% hydrogen, 7.5% carbon dioxide, and 5% oxygen when two evacuation (21 lb/in^2)-replacement cycles are completed. Both the need for a hydrogen-enriched atmosphere and the susceptibilities of many of the strains to antimicrobial agents typically contained in selective media must be considered when attempting to isolate *H. cinaedi* and *H. fennelliae* from clinical specimens.

In summary, we examined human and animal strains putatively identified as *H. cinaedi* and *H. fennelliae*. All but one strain identified as *H. cinaedi* by cellular fatty acid analysis were determined to be *H. cinaedi* by DNA-DNA hybridization. We were unable to differentiate this strain, D2084, from *H. cinaedi* isolates. In addition, we noted strains of *H. cinaedi* resistant to nalidixic acid and/or cephalothin, which may result in the misidentification of these clinical isolates. Both human *H. fennelliae* isolates were identified as *H. fennelliae* by DNA-DNA hybridization; however, neither animal isolate was *H. fennelliae*. True *H. fennelliae* strains are differentiated from the *H. fennelliae*-like strains by their abilities to grow in the presence of 1% glycine and their arylsulfatase activities.

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