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A Probable New *Helicobacter* Species Isolated from a Patient with Bacteremia

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A probable new *Helicobacter* species was isolated from the blood of a 14-month-old aboriginal child who presented with vomiting, diarrhea, fever, and dry cough. The most similar 16S rRNA gene sequence was that of *Helicobacter fennelliae* CCUG 18820^{T} but the new sequence differed from it by at least 32 base substitutions and by the presence of a large (353-nucleotide) intervening sequence.

Case report. A 14-month-old aboriginal child presented with a 1-week history of vomiting, diarrhea, fever, and dry cough. On admission, his temperature was 38°C and he had shortness of breath, rhinorrhoea, a discharge from the right ear, and crepitations on the left base of the lung. An ear swab grew *Pseudomonas aeruginosa* and *Streptococcus faecalis*. Lumbar puncture showed the cerebrospinal fluid was clear, and culture was negative. Urine collected from the patient was consistent with *Enterococcus faecalis* urinary tract infection. Hematological investigations showed eosinophilia, neutrophil leukocytosis, toxic granulation, and atypical lymphocytosis. A blood culture taken on admission grew a spiral gram-negative rod resembling *Campylobacter* species. X-rays taken at admission suggested a left lower lobe pneumonia. The organism was not isolated from the patient's feces or from his sputum (although incubation conditions may not have been ideal for the isolation of this organism). The patient was treated with intravenous ceftriaxone for 3 days (500 mg daily), with clinical improvement, and was discharged with a 1-week supply of oral amoxicillin (125 mg three times a day).

The organism was recovered from the inoculated aerobic bottle of the BACTEC Blood Culture System (Becton Dickinson Microbiology Systems, Cockeysville, Md.) after 6 days of incubation. Since the Gram stain indicated a *Campylobacter* sp., the bottle fluid was plated onto 6% sheep blood agar, chocolate agar, and a *Campylobacter* selective medium (Oxoid, Basingstoke, Hampshire, England). Plates were incubated in a microaerobic atmosphere (Campy Pak; Oxoid). The isolate recovered from blood culture grew on all three media at 37°C but failed to grow at 42°C and grew poorly at 25°C (microaerobic conditions, 3 days of incubation). It grew better in an H_2-enhanced microaerophilic atmosphere (10% H_2 and 6% CO_2 in N_2). Colonies were pinpoint, greyish white, and hemolytic on blood agar plates after several days of incubation. Biochemical tests (25) showed the organism to be oxidase and catalase positive but negative for nitrate reduction, sodium hippurate, and urea hydrolysis. The isolate was resistant to nalidixic acid (30-μg disk) and sensitive to cephalothin (30-μg disk), with a zone size of 21 mm if 6% horse blood agar was used. Media containing lysed blood (e.g., chocolate agar) did not give inhibition zones around disks. Sensitivity to nalidixic acid and cephalothin is used widely to distinguish between species of *Campylobacter* and *Helicobacter*, but, as demonstrated by this strain, the test results depended on the type of media used. The isolate (laboratory designation, strain VIDRL 6606) has been deposited in the American Type Culture Collection and given the designation ATCC 700956.

DNA from the plate culture was extracted and purified (33, 37), amplification of the 16S rRNA gene was performed using consensus terminal primers, and the amplified gene (~1,500 nucleotides [nt]) was sequenced directly using terminal and internal primers (15). A total of 1,825 unambiguous nucleotides were obtained. The larger-than-normal size of the gene was due to the presence of a 353-nt AT-rich intervening sequence (IVS) (or transcribed spacer). 16S rRNA sequences were obtained from GenBank or the Ribosome Database Project (http://www.cme.msu.edu/RDP/html) and aligned using the AE2 editor implemented at the Australian National Genomic Information Service (http://mel1.angis.org.au). After removal of common gaps, positions with uncertain bases, and terminal regions (where the sequences vary in length), 1,412 positions remained to use for tree reconstruction. The results of phylogenetic tree reconstructions (PAUP version 4.04b4a) (30) using the 16S rRNA gene sequences are shown in Fig. 1. The isolate branched within the helicobacter group (epsilon subdivision of *Proteobacteria*) and formed part of a statistically well-supported clade that included two other organisms, both originally isolated from AIDS patients. It was most closely related to a partial sequence of *Helicobacter fennelliae* (CCUG 18820^{T}) but differed from it by at least 32 base substitutions as well as by the presence of the IVS. This is a minimum estimate because the *H. fennelliae* sequence (accession number M88154) has 42 unresolved nucleotides, including one region where the number of bases is uncertain. The next closest 16S rRNA sequence (*Helicobacter* sp. strain Mainz) was less than 97% similar. Neither the *H. fennelliae* nor the *Helicobacter* sp. strain Mainz sequence contained an IVS. Using the guidelines proposed by Devereux et al. (6) and Stackebrandt and Goebel (26), a level of 97% or less sequence similarity in the 16S rRNA genes of two organisms would indicate they belong to separate species. We believe the evidence favors the classification of our isolate as a novel species of *Helicobacter*.

The IVS showed significant similarity to an IVS in a 23S rRNA sequence of *Helicobacter canis* NCTC 12743, a species that belongs to a separate clade (Fig. 1). The orientations of the two IVSs differed with respect to their surrounding rRNA

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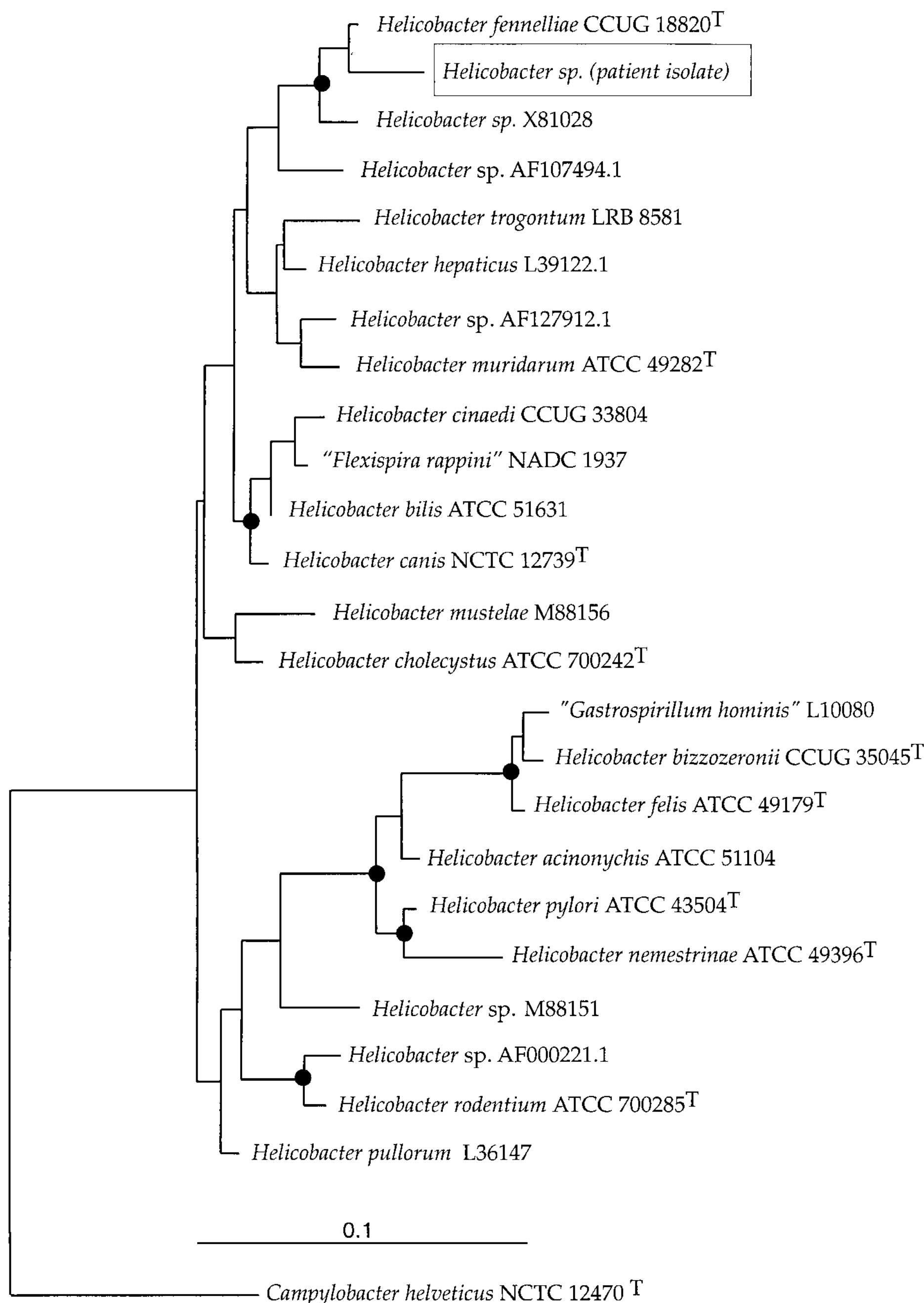

FIG. 1. Phylogenetic tree reconstruction using 16S rRNA gene sequences. The tree shown was produced by the maximum-likelihood method as implemented in PAUP 4.04b (30). Distance matrix and parsimony trees gave similar results but differed in the deepest branches. Bacterial names are followed by either culture collection numbers or GenBank accession numbers. Filled circles at branch points represent significant bootstrap values (≥75%) derived from the consensus distance matrix tree (100 replicates). The scale bar represents 0.1 expected substitutions per site.

genes. Secondary-structure predictions (mfold) (38) indicated that the new IVS can form a highly stable cloverleaf structure ($\Delta G = -128$ kcal/mol) with a primary stem of 16 nt (formed between the inverted terminal repeats of the IVS) and considerable base pairing elsewhere. The presence of an IVS in the 16S rRNA gene of this isolate is not surprising, as these elements occur in the 16S and 23S rRNA genes of many other bacteria (references 13 and 18 and references cited therein). We believe that our isolate shows the first example of an IVS that has a homologue in a different rRNA gene (of another organism) and where the two are in opposite orientations with respect to their surrounding genes. It is difficult to imagine a simple model of IVS transfer between organisms that would account for this pattern (18).

On the basis of the type of disease, *Helicobacter* spp. can be divided into three distinct groups. The first consists of gastric helicobacters such as *Helicobacter pylori* and *Helicobacter heilmanii*, which cause upper gastrointestinal tract diseases in humans. The second group consists of *Helicobacter* spp. that are isolated mainly from animals such as ferrets (11), rodents (16),

dogs (10, 28), cats (9), cheetahs (7), monkeys (3), and other mammals and have been found to cause disease in their respective hosts. The third group consists of helicobacters that cause enteric and systemic diseases in humans, such as *H. cinaedi* (4, 19–22, 32, 34), *H. fennelliae* (19, 34), "*H. westmeadii*" (35), *H. rappini* (1, 23, 24, 31), *Helicobacter* sp. strain Mainz (8, 14), *H. pullorum* (5, 27, 29), *Helicobacter* sp. strain CLO3 (21), and a number of unnamed species in GenBank, such as two strains isolated from AIDS patients in North America (36). All were recovered from the blood of patients except *H. pullorum*, and most were isolated from immunosuppressed persons. The reservoir in this group is unknown. Poultry are a likely source of food-borne enteritis in humans (2, 27), and *H. cinaedi* has been found in hamsters (12). Domestic animals like dogs and cats are also possible sources, and recently, two reports implied that dogs might be the reservoir for *H. rappini* infection (23, 31). Dogs are commonly found in and live in close proximity with aboriginal communities in Australia.

Nucleotide sequence accession number. The sequence determined in this study has been deposited in GenBank under accession number AF237612.

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REFERENCES

1. **Archer, J. R., S. Romero, A. L. Ritchie, M. E. Hamacher, B. M. Steiner, J. H. Bryner, and R. F. Schell.** 1988. Characterization of an unclassified microaerophilic bacterium associated with gastroenteritis. J. Clin. Microbiol. **26:**101–105.
2. **Atabay, H. I., J. E. L. Corry, and S. L. On.** 1998. Identification of unusual *Campylobacter*-like isolates from poultry products as *Helicobacter pullorum*. J. Appl. Microbiol. **84:**1017–1024.
3. **Bronsdon, M. A., C. S. Goodwin, L. I. Sly, T. Chilvers, and F. D. Schoenknecht.** 1991. *Helicobacter nemestrinae* sp. nov., a spiral bacterium found in the stomach of a pigtailed macaque (*Macaca nemestrina*). Int. J. Syst. Bacteriol. **41:**148–153.
4. **Burman, W. J., D. L. Cohn, R. R. Reves, and M. L. Wilson.** 1995. Multifocal cellulitis and monoarticular arthritis as manifestations of *Helicobacter cinaedi* bacteremia. Clin. Infect. Dis. **20:**564–570.
5. **Burnens, A. P., J. Stanley, R. Morgenstern, and J. Nicolet.** 1994. Gastroenteritis associated with *Helicobacter pullorum*. Lancet **344:**1569–1570.
6. **Devereux, R., S.-H. He, C. L. Doyle, S. Orkland, D. A. Stahl, J. LeGall, and W. B. Whitman.** 1990. Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. J. Bacteriol. **172:**3609–3619.
7. **Eaton, K. A., F. E. Dewhirst, M. J. Radin, J. G. Fox, B. J. Paster, S. Krakowka, and D. R. Morgan.** 1993. *Helicobacter acinonyx* sp. nov., isolated from cheetahs with gastritis. Int. J. Syst. Bacteriol. **43:**99–106.
8. **Fleisch, F., A. Burnens, R. Weber, and R. Zbinden.** 1998. *Helicobacter* species strain *Mainz* isolated from cultures of blood and from two patients with AIDS. Clin. Infect. Dis. **26:**526–527.
9. **Foley, J. E., S. L. Marks, L. Munson, A. Melli, F. E. Dewhirst, S. L. Yu, Z. L. Shen, and J. G. Fox.** 1999. Isolation of *Helicobacter canis* from a colony of Bengal cats with endemic diarrhea. J. Clin. Microbiol. **37:**3271–3275.
10. **Fox, J. G., R. Drolet, R. Higgins, R. Messier, L. Yan, B. E. Coleman, B. J. Paster, and F. E. Dewhirst.** 1996. *Helicobacter canis* isolated from a dog liver with multifocal necrotizing hepatitis. J. Clin. Microbiol. **34:**2479–2482.
11. **Fox, J. G., E. B. Cabot, N. S. Taylor, and R. Laraway.** 1988. Gastric colonization by *Campylobacter pylori* subsp. *mustelae* in ferrets. Infect. Immun. **56:**2994–2996.
12. **Gebhart, C. J., C. L. Fennell, M. P. Murtaugh, and W. E. Stamm.** 1989. *Campylobacter cinaedi* is normal, intestinal flora in hamsters. J. Clin. Microbiol. **27:**1692–1694.
13. **Hurtado, A., J. P. Clewley, D. Linton, R. J. Owen, and J. Stanley.** 1997. Sequence similarities between large subunit ribosomal RNA gene intervening sequences from different *Helicobacter* species. Gene **194:**69–75.
14. **Husmann, M., C. Gries, P. Jehnichen, T. Woelfel, G. Gerken, W. Ludwig, and S. Bhakdi.** 1994. *Helicobacter* sp. strain Mainz isolated from an AIDS patient with septic arthritis: case report and non-radioactive analysis of 16S rRNA sequence. J. Clin. Microbiol. **32:**3037–3039.
15. **Lane, D. J.** 1991. 16/23S rRNA sequencing, p. 115–175. *In* E. Stackenbrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley and Sons Inc., New York, N.Y.
16. **Lee, A., M. W. Phillips, J. L. O'Rourke, B. J. Paster, F. E. Dewhirst, G. J. Fraser, J. G. Fox, L. I. Sly, P. J. Romaniuk, T. J. Trust, and S. Kouprach.** 1992. *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. **42:**27–36.
17. **McNulty, C. A., J. C. Dent, A. Curry, J. S. Uff, G. A. Ford, M. W. Gear, and S. P. Wilkinson.** 1989. New spiral bacterium in gastric mucosa. J. Clin. Pathol. **42:**585–591.
18. **Miller, W. L., K. Pabbaraju, and K. E. Sanderson.** 2000. Fragmentation of 23S rRNA in strains of *Proteus* and *Providencia* results from intervening sequences in the *rrn* (rRNA) genes. J. Bacteriol. **182:**1109–1117.
19. **Ng, V. L., W. K. Hadley, C. L. Fennell, B. M. Flores, and W. E. Stamm.** 1987. Successive bacteremias with "*Campylobacter cinaedi*" and "*Campylobacter fennelliae*" in a bisexual male. J. Clin. Microbiol. **25:**2008–2009.
20. **Orlicek, S. L., D. F. Welch, and T. L. Kuhls.** 1993. Septicemia and meningitis caused by *Helicobacter cinaedi* in a neonate. J. Clin. Microbiol. **31:**569–571.
21. **Pasternak, J., R. Bolivar, R. L. Hopfer, V. Fainstein, K. Mills, A. Rios, G. P. Bodey, C. L. Fennell, P. A. Totten, and W. E. Stamm.** 1984. Bacteremia caused by *Campylobacter*-like organisms in two male homosexuals. Ann. Intern. Med. **101:**339–341.
22. **Quinn, T. C., S. E. Goodell, C. Fennell, S.-P. Wang, M. D. Schuffler, K. K. Holmes, and W. E. Stamm.** 1984. Infections with *Campylobacter jejuni* and *Campylobacter*-like organisms in homosexual men. Ann. Intern. Med. **101:** 187–192.
23. **Romero, S., J. R. Archer, M. E. Hamacher, S. M. Bologna, and R. F. Schell.** 1988. Case report of an unclassified microaerophilic bacterium associated with gastroenteritis. J. Clin. Microbiol. **26:**142–143.
24. **Schauer, D. B., N. Ghori, and S. Falkow.** 1993. Isolation and characterization of "*Flexispira rappini*" from laboratory mice. J. Clin. Microbiol. **31:**2709–2714.
25. **Smibert, R. M., and N. R. Krieg.** 1994. Phenotypic characterization, p. 607–654. *In* P. Gerhardt (ed.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
26. **Stackebrandt, E., and B. M. Goebel.** 1994. A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44:**846–849.
27. **Stanley, J., D. Linton, A. P. Burnens, F. E. Dewhirst, S. L. On, A. Porter, R. J. Owen, and M. Costas.** 1994. *Helicobacter pullorum* sp. nov.—genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. Microbiology **140:**3441–3449.
28. **Stanley, J., D. Linton, A. P. Burnens, F. E. Dewhirst, R. J. Owen, A. Porter, S. L. On, and M. Costas.** 1993. *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. J. Gen. Microbiol. **139:**2495–2504.
29. **Steinbrueckner, B., G. Haerter, K. Pelz, S. Weiner, J.-A. Rump, W. Deissler, S. Bereswill, and M. Kist.** 1997. Isolation of *Helicobacter pullorum* from patients with enteritis. Scand. J. Infect. Dis. **29:**315–318.
30. **Swofford, D. L.** 2000. PAUP (and other methods): Phylogenetic Analysis Using Parsimony, version 4. Sinauer Associates, Sunderland, Mass.
31. **Tee, W., K. Leder, E. Karroum, and M. Dyall-Smith.** 1998. "*Flexispira rappini*" bacteremia in a child with pneumonia. J. Clin. Microbiol. **36:**1679–1682.
32. **Tee, W., A. C. Street, D. Spelman, W. Munckhof, and A. Mijch.** 1996. *Helicobacter cinaedi* bacteremia: varied clinical manifestations in three homosexual males. Scand. J. Infect. Dis. **28:**199–203.
33. **Tee, W., M. Dyall-Smith, W. Woods, and D. Eisen.** 1996. Probable new species of *Desulfovibrio* isolated from a pyogenic liver abscess. J. Clin. Microbiol. **34:**1760–1764.
34. **Totten, P. A., C. L. Fennell, F. C. Tenover, J. M. Wezenberg, P. L. Perine, W. E. Stamm, and K. K. Holmes.** 1985. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennelliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. J. Infect. Dis. **151:**131–139.
35. **Trivett-Moore, N. L., W. D. Rawlinson, M. Yuen, and G. L. Gilbert.** 1997. *Helicobacter westmeadii* sp. nov., a new species isolated from blood cultures of two AIDS patients. J. Clin. Microbiol. **35:**1144–1150.
36. **Weir, S. C., C. L. Gibert, F. M. Gordin, S. H. Fischer, and V. J. Gill.** 1999. An uncommon *Helicobacter* isolate from blood: Evidence of a group of *Helicobacter* spp. pathogenic in AIDS patients. J. Clin. Microbiol. **37:**2729–2733.
37. **Wilson, K.** 1990. Preparation of genomic DNA from bacteria, supplement 24, p. 2.4.1–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley and Sons, New York, N.Y.
38. **Zuker, M., D. H. Mathews, and D. H. Turner.** 1999. Algorithms and thermodynamics for RNA secondary structure prediction, p. 11–43. *In* J. Barciszewski and B. F. C. Clark (ed.), A practical guide in RNA biochemistry and biotechnology. NATO ASI Series. Kluwer Academic Publishers, Dordrecht, The Netherlands.