

## “*Helicobacter rappini*” Isolates from 2 Homosexual Men

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**We report 2 cases of bacteremia due to “*Helicobacter rappini*” in 2 young, homosexual men, including the first report of *H. rappini* in a human immunodeficiency virus–positive patient. Blood cultures showed a spiral, fusiform, gram-negative bacterium with bipolar sheathed flagella.**

“*Helicobacter rappini*,” a gram-negative, motile, fusiform bacterium with periplasmic fibers and bipolar tufts of sheathed flagella, has previously been known as “*Flexispira rappini*.” Both names are currently without formal taxonomic status (i.e., they are not on the lists of approved bacterial names), but phylogenetically this organism is closely related to the genus *Helicobacter*, and it shares morphological features with some *Helicobacter* species, such as *Helicobacter bilis* [1], *Helicobacter canis* [2], and *Helicobacter trogontum* [3]. *H. rappini* has been isolated from various animal sources, including aborted sheep fetuses [4, 5], the intestinal mucosa of laboratory mice [6], and stool samples obtained from puppies [7]. Reports of infections due to this organism in humans were previously uncommon, but with improved blood culture systems and the use of more-advanced molecular methods (e.g., 16S rRNA gene sequencing) during the past 2 years, the Victorian Infectious Diseases Reference Laboratory in North Melbourne, Australia, has been able to detect 4 such cases, 1 of which has been described elsewhere [8].

**Patient 1.** A 26-year-old HIV-infected man had a history of AIDS, including cerebral toxoplasmosis, candidiasis, chronic anemia, nephrotic syndrome, and HIV-related cardiomyopathy

that caused biventricular failure (New York Heart Association grade IV). He had an HIV RNA load of 272,000 copies/mL and a CD4 cell count of  $13 \times 10^9$  cells/ $\mu$ L. He lived on a small farm where horses and dogs also resided, but he had not traveled overseas in the recent past. His medications included efavirenz, stavudine, and lamivudine but not trimethoprim-sulfamethoxazole.

The patient presented to the emergency department of his local hospital with fever (temperature, 38°C), tachycardia, diarrhea, and signs of right and left heart failure. Investigations revealed mild neutrophilia, but the results of fecal microscopy and routine cultures were not diagnostic. Treatment with captopril, furosemide, and oxygen administered by mask resulted in a good initial effect, and the patient then was transferred to Alfred Hospital, Prahran, Australia. His temperature normalized, but after 5 days of incubation, cultures of blood samples obtained at the time of admission yielded a gram-negative, spiral, fusiform rod that was identified as *H. rappini*. He was then treated with a 2-week course of oral ciprofloxacin, and the diarrhea resolved. He was discharged to his home to receive palliative care, and he died of progressive cardiac and renal failure 6 weeks later. During this time, there was no recurrence of fever or diarrhea.

**Patient 2.** A 20-year-old homosexual man presented with a 2-week history of fever, malaise, and pharyngitis and with a 24-h duration of nausea, vomiting, and diarrhea. He had a past history of perianal herpes simplex virus type II infection, gonorrhea, asthma, and depression. He had not traveled overseas and had not had contact with animals; however, he had helped to care for a number of elderly people 10 days earlier. On examination, dehydration, a temperature of 37.5°C, and generalized abdominal tenderness and hyperactive bowel sounds were noted. Investigation revealed the presence of WBCs in his feces, but routine cultures revealed no pathogens. Full blood examination showed marked leukocytosis (WBC count,  $27.8 \times 10^9$  cells/L; normal range for WBC count,  $3.5 \times 10^9$  cells/L to  $9.5 \times 10^9$  cells/L) with a left shift and toxic granulation. The results of renal and liver function tests were normal, and the patient tested negative for HIV antibody. On the day of admission to the hospital, culture of a blood sample that had been obtained by his local doctor 5 days previously yielded positive results, and microscopy revealed a spiral, fusiform, gram-negative rod, which was later identified as *H. rappini*. He was treated with a 2-week course of oral ciprofloxacin, and his fevers and diarrhea abated within 24 h after initiation of therapy.

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\* Dr. Wee Tee died on 14 August 2000, after a long illness. She was a highly respected microbiologist and will be greatly missed as both friend and colleague.

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**Materials and methods.** The 2 strains of *H. rappini* were recovered from the inoculated bottles of the BacT/Alert Microbial Detection System (Organon Teknika) and VITAL AER (bioMérieux) after 5 days of incubation. Bottles were subcultured onto 6% sheep blood agar plates and were incubated in an H<sub>2</sub>-enhanced microaerobic atmosphere, and the cultures were examined daily for growth.

Biochemical testing for *Helicobacter* species, Gram smear morphologic testing, and phase-contrast microscopy were done by use of standard methods. Antibiotic susceptibility tests were done using a modification of the agar disk diffusion method, in which 6% horse blood agar plates and a heavy inoculum of organisms (0.1 mL of 10<sup>9</sup> cells/mL, as estimated by use of MacFarland standards) were used.

For examination by electron microscopy, the sample was prepared by means of agar diffusion onto Formvar-coated (Sigma Chemical) copper grids. They were stained with 2.0% ammonium molybdate and were examined by use of an electron microscope (Phillips CM10) that operated at 60 kV.

DNA preparation, 16S rRNA gene amplification, and gene sequencing were done according to the procedure described elsewhere [9]. 16S rRNA gene analysis identified the strains as *H. rappini*. The sequences have been deposited in GenBank (GenBank accession numbers AF286052 and AF286053).

**Results.** Both strains of *H. rappini* were successfully isolated in an H<sub>2</sub>-enhanced microaerobic atmosphere. Microscopy showed them to be slightly spiral, fusiform, gram-negative rods (4–5 μm × 0.5 μm) that sometimes showed a winged (end-to-end) arrangement of 2 cells. Electron microscopy of the isolate that was recovered from patient 1 also demonstrated bipolar sheathed flagella (figure 1), a feature that is characteristic of this genus. Biochemical tests showed positive reactions in catalase and oxidase but negative reactions in sodium hippurate and urea hydrolysis. Antimicrobial disk susceptibility testing showed that the 2 strains were resistant to both cephalothin and nalidixic acid. Disk diffusion tests showed that one of the strains (which was isolated from the non-HIV-infected patient) was susceptible to ciprofloxacin, doxycycline, gentamicin, ceftriaxone, imipenem, amoxicillin-clavulanate, and ticarcillin/clavulanate but resistant to erythromycin, ceftazidime, and cefotaxime. The Etest (AB Biodisk) was used by the original laboratory to test for susceptibility to ciprofloxacin, and the isolate was found to be susceptible (MIC, 0.032 μg/mL).

The nearly complete 16S rRNA sequences of the 2 isolates (1453 and 1486 nucleotides, respectively) differed from each other by only 35 base changes (2.4%). The closest relatives of both isolates were among reported *H. rappini* isolates (difference in sequences, ≤25 nucleotides [≤1.7%]) or to unpublished isolates with sequences very similar to that of *H. rappini*. The sequence closest to that of the isolate recovered from patient 1 (GenBank accession number AF023862; difference in



**Figure 1.** Negative-stain electron micrograph of an isolate of “*Helicobacter rappini*” recovered from an HIV-infected patient (patient 1) showing a thin, spiral cell with sheathed polar flagellum. Bar denotes 1 μm.

sequences, 6 nucleotides) was that of an unpublished isolate recovered from the blood of a Canadian patient with AIDS. The next closest relative (difference in sequences, 16 nucleotides) was an *H. rappini* isolate that was recovered from the blood of a child with pneumonia [8], and this isolate was also the closest relative (difference in sequences, 25 nucleotides) to the isolate recovered from patient 2.

**Discussion.** Two main groups of *Helicobacter* species are associated with diseases in humans. The first group consists of gastric *Helicobacter* species, such as *Helicobacter pylori* [10] and *Helicobacter heilmannii* [11, 12], which cause upper gastrointestinal diseases in humans. The second group consists of *Helicobacter* species that cause enteric and systemic diseases. This group includes such species as *Helicobacter cinaedi* [13–16], *Helicobacter fennelliae* [16, 17], *Helicobacter westmeadii* [18], *H. rappini* [7, 8, 19], *Helicobacter* species strain Mainz [20, 21], *Helicobacter pullorum* [22–24], *Helicobacter canadensis* [25], and a number of unnamed species with sequences deposited in the GenBank database, such as strains isolated from patients with AIDS in Vancouver, Canada, and Washington, DC [26]. All of these species, with the exception of *H. pullorum*,

have been recovered from blood samples obtained from patients with bacteremia. Most of these patients had immunosuppression, with the majority being infected with HIV. As far as we know, the presence of *H. rappini* in an HIV-infected patient has not been previously reported.

Since the first published reports of the isolation of *H. rappini* from the feces of humans with gastroenteritis [7, 19], *H. rappini* has been isolated from patients' blood samples in only 3 published case reports, which described a 9-year-old child with pneumonia [8], a 65-year-old febrile man who was undergoing hemodialysis [27], and a patient with agammaglobulinemia [28], respectively. The organism has also been isolated from bile samples obtained from 2 Chileans with chronic cholecystitis [29]. *H. rappini* appears to be able to invade the bloodstream of a wide range of hosts, from previously healthy persons to patients who are immunocompromised.

Previous reports have shown that strains of *H. rappini* that were isolated from humans tested positive for urea hydrolysis [7, 19]; however, both of our isolates tested negative for urea hydrolysis, despite repeated testing. An *H. rappini*-like organism recovered from cotton-top tamarins with colitis was reported to test negative for urease [30]. The fastidious nature and the slow growth of the organism make biochemical tests unreliable or of limited use, and often misidentification can occur. Susceptibility to nalidixic acid and cephalothin is part of the criteria for identification of *Campylobacter* and *Helicobacter* species, but the test needs to be interpreted with care, because any prior exposure to these antibiotics may affect the results, especially among patients who are treated with antibiotics. Molecular techniques, such as 16S rRNA gene sequence analysis, have been shown to be successful for the identification and differentiation of species in this genus.

The reservoir in this group is thus far unknown. Some studies have suggested that *H. cinaedi* has been found in hamsters in the United States [31]. Poultry products appeared to be a likely source of foodborne enteritis [24] associated with *H. pullorum* in humans. Domestic animals, such as dogs and cats, that have close contact with humans also are possible sources [7, 19]. One of our patients lived on a farm and had direct contact with horses and dogs.

The method of antimicrobial susceptibility testing used for the non-*H. pylori* group of *Helicobacter* species has not been standardized, and evaluation of in vivo and in vitro susceptibility has not been done. Given these uncertainties, it is difficult to provide adequate information to clinicians regarding the best choice of antibiotics to use in treating this group of blood-associated *Helicobacter* infections. In both of our patients, the use of ciprofloxacin appeared to resolve the symptoms. However, we know that quinolone-resistant strains were detected in HIV-infected patients with *Campylobacter* infections [32] and, also, in patients with infections associated with *H. cinaedi* [14,

15, 33]. Among patients with infections due to *H. cinaedi*, in vitro resistance to ciprofloxacin and erythromycin appeared to correlate with treatment failure in patients who were treated with these antibiotics. Other agents that may be useful for the treatment of "*H. rappini*" include gentamicin, doxycycline, imipenem, amoxicillin/clavulanate, and ticarcillin/clavulanate.

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