Helicobacter westmeadii sp. nov., a new species isolated from blood cultures of two AIDS patients.

N L Trivett-Moore, W D Rawlinson, M Yuen and G L Gilbert

Helicobacter westmeadii sp. nov., a New Species Isolated from Blood Cultures of Two AIDS Patients

N. L. TRIVETT-MOORE,* W. D. RAWLINSON, M. YUEN, AND G. L. GILBERT
Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead 2145 New South Wales, Australia

Received 26 July 1996/Returned for modification 30 September 1996/Accepted 11 February 1997

A slowly growing anaerobic Helicobacter species was isolated from the blood cultures of two human immunodeficiency virus-positive patients admitted to Westmead Hospital, Westmead, Australia, with fevers. The morphology of the isolates was consistent with Helicobacter cinaedi or Helicobacter fennelliae. The results of culture growth conditions, biochemical tests, gas chromatography data, ribotyping, and 16S rDNA sequencing showed that these isolates represent a new Helicobacter species, for which the name Helicobacter westmeadii has been proposed.

The clinical importance of Helicobacter species as gastrointestinal and systemic pathogens in humans has increased in the past decade. The type species of this genus, Helicobacter pylori, causes chronic gastritis and peptic ulcer disease in humans (11, 19) and is now linked to the development of gastric adenocarcinoma and gastric mucosa-associated lymphoma (22, 40). Other helicobacters of clinical interest are Helicobacter pylori, Helicobacter pullorum, and Helicobacter suis. They have been isolated from the blood of febrile and neutropenic patients with fever and gastroenteritis (3, 7, 28, 29).

Although initially associated with gastroenteritis in homosexual men (12, 25), H. cinaedi and H. fennelliae have also been isolated from asymptomatic (25) and bacteremic (3) human immunodeficiency virus (HIV)-positive and -negative homosexual males. H. cinaedi alone has been isolated from a symptomatic heterosexual male (12), a symptomatic female, and a child presenting with acute diarrheal gastroenteritis (36). It has also been isolated from an asymptomatic female as well as a group of children who presented with no signs of clinical illness (36). H. cinaedi and H. fennelliae were originally isolated from rectal swabs taken from homosexual males in the early 1980s (7) and were first thought to be fastidious Campylobacter-like organisms. They were initially named Campylobacter cinaedi and Campylobacter fennelliae (35). However, Thompson et al. (34) reported that they belonged to a single rRNA homology group separate from the genus Campylobacter; therefore, these isolates were placed into the genus Helicobacter by Vandamme et al. in 1991 (37).

This paper describes the clinical and laboratory findings in two cases of Helicobacter bacteremia from AIDS patients. We propose that the isolates represent a new species of Helicobacter related to H. cinaedi.

CASE REPORTS

Patient one. Patient one was a 33-year-old homosexual male who was found to be HIV antibody positive in 1985. During 1992 and 1993, he suffered from Pneumocystis carinii pneumonia, recurrent perianal herpes, cryptococcal meningoencephalitis, and Kaposi’s sarcoma, for which he was receiving chemotherapy. During this time, he developed oral candidiasis and microsporidiosis.

In January 1994, he was admitted to Westmead Hospital, Westmead, Australia, since he was febrile and neutropenic (total leukocyte count, 1.6 × 10⁹/liter; neutrophils, 0.6%) following an episode of chemotherapy. His medications upon admission were dapsone (100 mg daily), fluconazole (400 mg daily), and acyclovir (200 mg twice daily). A motile gram-negative rod was isolated from his blood culture (see Materials and Methods). Other cultures were negative, and all other investigations were unremarkable.

The patient was treated empirically with ticarcillin-clavulanic acid and tobramycin, and his fever gradually resolved coincidentally with the elevation of his leukocyte count. He died 11 months later with extensive Kaposi’s sarcoma.

Patient two. Patient two was a 28-year-old heterosexual male who was found to be HIV antibody positive in 1991. HIV-related complications began in late 1993 and included oral candidiasis, diarrhea, and subsequent weight loss accompanied by anxiety and depression.

The patient was admitted to Westmead Hospital in January 1994 with a 4-week history of cellullitis of the right leg. A blood culture was taken, from which a gram-negative rod was later isolated. He was treated for cellulitis with penicillin and fluclaxacin with no effect. During his hospital stay, the patient became febrile and developed a maculopapular rash, oral candidiasis, and a fungal infection in his toes. Treatment was changed to cephalothin, to which he initially responded; however, he later developed recurrent lesions on his legs, a biopsy of which showed nonspecific inflammatory changes. He subsequently recovered from this acute illness but died some months later of HIV-related illnesses.

These two patients lived within the same geographical region of Sydney, Australia, but not in the same suburb. They had had no known contact with each other, nor had they had any history of exposure to animals or recent travel.

MATERIALS AND METHODS

Blood collection. Blood for culture was collected from both patients by venipuncture. A 5- to 10-ml volume was inoculated into BACTEC Plus 26 (aerobic) and BACTEC Plus 27 (anaerobic) bottles (Becton Dickinson, Cockeysville, Md.), which were incubated in the BACTEC 460 instrument.

Culture. The aerobic blood culture media from both patients indicated growth after 6 days of incubation. A Gram stain was performed, and BACTEC fluid
from both anaerobic and aerobic culture bottles was subcultured onto blood agar (Columbia agar base [Oxoid, Basingstoke, United Kingdom] containing 5% horse blood [Micro Diagnostics, Brisbane, Australia]) incubated in air, MacConkey agar (GIBCO-BRL, Life Technologies, Paisley, Scotland) incubated in air, enriched anaerobic agar (Columbia agar base containing 5% horse blood) incubated in 5% CO2. When no growth was detected at 37°C on routine subculture, the BACTEC fluid was inoculated onto additional media, namely, blood agar containing 5% human fecal extract and fresh Trypticase soy agar (Becton Dickinson) incubated in 2% oxygen and postfixed in 2% osmium tetroxide (buffered in 0.1 M sodium cacodylate) for 4 h. The rods were dehydrated through an alcohol series and were critical point dried, mounted on stubs, and coated with gold. The mounted stubs were observed with a Hitachi field emission scanning EM.

### Biochemical, API ZYM, and diagnostic antibiotic susceptibility tests

Detailed biochemical characterization was performed for both isolates. Gram staining and tests for motility, urease, oxidase, catalase, and H2S production and nitrate reduction were performed by standard methods (2). Hippurate hydrolysis was tested by the method described by Moran et al. (17). Rapid carbohydrate utilization was determined with tablets from Rosco Diagnostics (Taastrup, Denmark) according to the manufacturer’s instructions. Susceptibility to antimicrobial agents was determined as described by Paster et al. (23). An API ZYM (Biomerieux, Marcyl’Etoile, France) rapid-test strip for each isolate was set up according to the manufacturer’s instructions and incubated for 4 h at 37°C in an aerobic atmosphere. All biochemical tests were performed in triplicate by different individuals at different times to test for reproducibility.

### Gas chromatography (GC) of cellular fatty acids

Fatty acids were analyzed with the MIDI automated microbial identification system (MIDI) as described by Springer et al. (27), except that a Hewlett-Packard model 5890 GC and a Hewlett-Packard 3900 computer were used. The Eucalanian distance was calculated using the MIDI software.

### DNA extraction for ribotyping and 16S rDNA sequencing

Bacteria were cultured on TSA-agar plates. Colonies were emulsified from the plates with a sterile loop and suspended in 5 ml of TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA [pH 8.0]) (pH 8.0). The bacteria were lysed by the addition of 1 ml of lysozomal solution (100 mM NaCl, 10 mM Tris-HCl, 25 mM EDTA, 0.5% sodium dodecyl sulfate, 0.1 mg proteinase K) and were incubated at 55°C overnight. DNA was extracted with phenol-chloroform-isooamylalcohol (25:24:1) as previously described (26). Ribotyping. An 8-µg amount of bacterial DNA was digested with 40 U of PstI restriction enzyme (New England Biolabs) at 37°C for 5 h. The isolates were characterized by hybridization with a 16S rDNA probe, which was produced by PCR from cDNA of the 16S plus 23S rDNA of Escherichia coli MRE 600 (Boehringer Mannheim, Castle Hill, New South Wales, Australia) as previously described (24). The 16S rDNA PCR probe was 476 bp in length and corresponded to Es. coli 16S rRNA positions 93 to 392, respectively. Electrophoresis, Southern blotting, and probing were all performed by the method described by van Embden et al. (38), with modifications.

### Amplification and purification of 16S rDNA

Approximately 1.5 kb of the gene encoding 16S rRNA was amplified with conserved primers (39). Approximately 100 ng of PCR-amplified bacterial DNA was visualized in a 0.7% agarose gel and purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI). The reaction mix consisted of 2 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% Tween 20, 0.01% gelatin, 0.01% Nonidet P-40, 10 pmol of each conserved universal primer, and 1 U of Dynazyme TG polymerase (Finnzymes Oy, Tampere, Finland). The reaction mix was overlaid with liquid paraffin, and the following conditions were used for amplification: initial denaturation at 96°C for 3 min and then five cycles of denaturation at 96°C for 45 s, annealing at 53°C for 30 s, and elongation at 72°C for 1 min. This protocol was then followed by 28 cycles of denaturation at 96°C for 45 s, annealing at 48°C for 30 s, and elongation at 72°C for 1 min followed by a final elongation step at 72°C for 10 min. PCR was performed in a Corbett FTS-320 thermal sequencer (Corbett Research, Mortlake, Australia). The purity of the amplified product was determined by electrophoresis in a 0.8% agarose gel (Amresco, Solon, Ohio) with ethidium bromide staining. The PCR product was viewed under UV light.

The amplified DNA was purified with polyethylene glycol 8000 precipitation as described by Fox et al. (9).

### 16S rDNA sequencing and analysis

The PCR product was sequenced with Applied Biosystems (AB1) Tag DyeDeoxy terminator cycle-sequencing kits according to standard protocols (4). Eighty forward and 70 reverse primers were used in separate linear amplification reactions to sequence both strands of the 1.5-kb 16S rDNA PCR product. The sequences were assembled with Staden software (5), and sequence alignment was performed with Clustal W (33). A phylogenetic tree was constructed by maximum parsimony methods (33).

### Nucleotide sequence accession number

The complete 16S rDNA consensus sequence has been deposited with GenBank under accession no. U44756.

### RESULTS

#### Isolation and growth characteristics

After 6 days of incubation, the aerobic BACTEC bottles from both patients gave positive results. However, the growth values indicated for both of these bottles were only 35, indicating poor growth. No growth was detected in the anaerobic bottles from both patients. Gram staining of the broth from both aerobic and anaerobic bottles originally showed no organisms present; however, dark-field microscopy revealed slowly moving spiral rods present in the aerobic bottles only. Neither isolate grew after routine subculture from either the aerobic or the anaerobic bottles from both patients. The isolates subsequently grew from the aerobic bottles on fresh TSA plates supplemented with 5% defibrinated HB after 4 days of anaerobic incubation at 37°C. The colonies were small, slightly mucoid, translucent, and swarming. A Gram stain revealed thin gram-negative spiral rods. Growth was seen under microaerophilic conditions at 37°C, but these conditions required 5 days of incubation to produce visible growth. Under these growth conditions, the bacteria lost their ability to swarm. Slight growth was observed in the brucella broth supplemented with 5% defibrinated HB after 4 days of anaerobic incubation at 37°C, but viability was difficult to sustain. No growth was observed with any other medium at 25, 37, or 42°C under anaerobic, microaerophilic, or aerobic conditions.

### Ultrastructure

Both isolates measured 0.5 by 1.5 to 2 µm. They were rod or spiral shaped, and each cell had a single, sheathed, unipolar flagellum. The basal body of the flagellum could be seen embedded within the membrane of the bacterium, as shown in Fig. 1. Cellular morphology resembled those of H. cinaedi and H. fennelliae. Figure 2 shows the appearance of the cells during division, when another flagellum appeared on the opposite end of the bacterium and a pit formed in the middle of the cell body, from which separation occurred.

### Biochemical, API ZYM, and diagnostic antibiotic susceptibility tests

The results of biochemical, API ZYM, and antibiotic susceptibility tests for both isolates are shown in Table 1. These were compared with other known Helicobacter species isolated from humans. Like H. cinaedi and H. fennelliae, both isolates were oxidative and catalase positive and urease negative, did not ferment or oxidize sugars, and did not produce indole or H2S. They were motile and gram negative, and, like H. cinaedi, they reduced nitrate to nitrite and were sensitive to nalidixic acid but were resistant to cephalexin. Unlike H. cinaedi and H. fennelliae, both isolates hydrolyzed hippurate.

The API ZYM rapid-test strip detects production of 11 different enzymes by bacteria. Both H. westmeadi isolates produced the same profile, giving results closest to those for _H. ecoli_.
pylori, which was the only Helicobacter species on the database (Table 1).

**GC of cellular fatty acids.** The results of separation of fatty acid methyl esters by gas-liquid chromatography with MIS are shown in Table 2. Both isolates produced the same profile. H. cinaedi was the closest match from the MIDI library database; however, the Euclidean distance gave a relatedness value of 11.4 between H. cinaedi and the two isolates. Values greater than 6 indicate organisms of a different species.

**Ribotyping.** The results of ribotyping are shown in Fig. 3. H. westmeadii isolates produced a pattern clearly different from those of both H. cinaedi ATCC 35683 and H. pylori ATCC 43504 type strains. The two H. westmeadii isolates demonstrated patterns similar to each other.
**TABLE 1.** Biochemical, API ZYM, diagnostic antibiotic susceptibility tests and growth characteristics of *H. westmeadii* and related bacteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>H. westmeadii</em></th>
<th><em>H. cinaedi</em></th>
<th><em>H. fennelliae</em></th>
<th><em>H. pylori</em></th>
<th><em>H. canis</em></th>
<th><em>H. pullorum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease activity</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H₂S production</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Resistance (30-μg disk)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>I</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth at (°C):</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>42</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth under:</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Aerobic conditions</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Microaerophilic conditions</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic conditions</td>
<td>+</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CS esterase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CS esterase lipase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>W</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Naphthol-AS-BI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Sources for these data, this study and references 2, 28, and 29. W, weak growth detected; I, intermediate; ND, not determined.

**DISCUSSION**

The isolates from our patients were very similar to *H. cinaedi*, both morphologically and biochemically. They could have been mistaken for either *H. cinaedi* or *H. fennelliae*, both of which have been isolated from blood and gastrointestinal specimens of HIV-positive homosexual males (3, 7, 12, 35). *H. cinaedi* has also been isolated from a stool sample of a symptomatogenic heterosexual male (12). As previously reported for *H. cinaedi* (3, 16), our isolates could not initially be seen upon Gram staining of BACTEC medium. They were also isolated by using Clustal W (Fig. 4). *H. westmeadii* falls into a cluster which includes *H. cinaedi*, *H. bilis*, and *Flexispira rappini*.

![Ribotyping patterns from *H. westmeadii* isolates, *H. cinaedi* ATCC 35683, and *H. pylori* ATCC 43504. Molecular weight markers (MW) are in kilobases. Lanes: 1, *H. pylori* type strain; 2, *H. cinaedi* type strain; 3, and 4, *H. westmeadii* isolate from patients one and two, respectively.](http://jcm.asm.org/35/3/1147)
only from aerobic BACTEC medium, as is the case for all but one *H. cinaedi* isolate. However, their growth in this medium was poor; therefore, we can assume that they were only being sustained by this medium. This is supported by the difficulty we had in recovering the isolates on subculture from this medium.

*Helicobacter* and *Campylobacter* species are generally described as microaerophilic (2, 17) and prefer an atmosphere of 7 to 12% carbon dioxide, 0 to 85% hydrogen, and 0 to 85% nitrogen. Their intolerance of atmospheric oxygen is due to their sensitivity to superoxides and free radicals (2), despite the possession of catalase and superoxide dismutase enzymes. The addition of whole blood to growth medium neutralizes oxygen and reduces the toxicity of these molecules. *H. westmeadii* grew relatively slowly and lost motility in a microaerophilic environment and did not grow at all in an aerobic environment. This may be because *H. westmeadii* has a sensitivity to oxygen greater than those of other *Helicobacter* species. This fact may also explain the difficulty in culturing this species from aerobic BACTEC medium. However, it is not clear why this species apparently failed to survive or grow in anaerobic BACTEC medium.

The preference for anaerobic growth conditions appears to be a unique characteristic of *H. westmeadii*. Kiehlbauch et al. (15) reported that *H. cinaedi* and *H. fennelliae* would grow under anaerobic conditions but suggested that this was due to laboratory adaption of their strains to a “controlled anaerobic environment.” All of their anaerobic strains failed to hydrolyze hippurate and thus differed from our isolates.

The ability to hydrolyze hippurate was the most useful biochemical characteristic that distinguished *H. westmeadii* species from *H. cinaedi*, *H. fennelliae*, and other *Helicobacter* species. Although *Campylobacter jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei* also hydrolyze hippurate, *H. westmeadii* was distinguished from campylobacters on the basis of growth re-

FIG. 4. Phylogenetic tree for the helicobacters, campylobacters, and related organisms. The tree was rooted with *Bacteroides ureolyticus* and *E. coli* as outgroups.
The natural reservoir of most *Helicobacter* and *Campylobacter* species is the intestinal tracts of animals. These bacteria have acquired a specialized motility that allows them to inhabit the intestinal mucus. Despite the presence of a similar ecological niche in humans, these bacteria rarely appear in the human intestinal tract, although there have been suggestions that they are more common in some populations (20). The isolation of the bacteria reported here brings the number of species isolated from homosexual or HIV-positive patients to four. One explanation is that these types of bacteria are carried by a larger proportion of persons and become apparent only with immunosuppression, probably via translocation from the lower bowel mucus. The recent isolation of *Helicobacter hepaticus* and its association with hepatitis and hepatocellular carcinoma (8) are likely to increase interest in this group of bacteria.

The source of infection of our patients is not known. They had no known contact with each other, but they did live in the same geographical region with a common water supply. An infection with *H. cinaedi* which may have originated from a contaminated water supply has been reported elsewhere (16). Animal-to-human transmission cannot be ruled out, since infection with *H. cinaedi* in this way has been suggested (10). However, our patients had had no known contact with animals before their hospital admission. Sexual practices involving multiple partners and oral-anal contact (25) are the most likely modes of transmission, although the epidemiologic association of *H. cinaedi* with homosexual men has not been satisfactorily explained.

The biochemical and phylogenetic characteristics of *H. westmeadii* suggest that we have isolated a new species of *Helicobacter* from blood cultures of two immunocompromised HIV-infected males. *H. westmeadii* apparently did not cause severe disease in these patients; its origin is unknown.

### Description of *H. westmeadii* sp. nov.

*H. westmeadii* is a gram-negative bacterium that is either rod or spiral shaped, 0.5 μm in diameter, and 1.5 to 2 μm in length. The cells are motile, with a smooth forward motion, and each cell has a single, unipolar, sheathed flagellum. During cell division, another polar flagellum forms on the opposite end of the cell and a pit-like structure appears in the middle of the cell where division occurs. The cells are anaerobic but will tolerate a microaerophilic environment with a retarded growth rate. Viability is rapidly lost in air. Growth is best on fresh, moist TSA supplemented with 5% HB incubated at 35 to 37°C. The culture grows as a fine, translucent, spreading film after 4 days of incubation. Alkaline phosphatase, C4 esterase, C8 esterase, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase are produced. There is no production of lysine decarboxylase, ornithine decarboxylase, or arginine decarboxylase. No acid is produced from glucose, xylose, mannitol, lactose, sucrose, maltose, or fructose. Oxidase and catalase are present, but urease is absent, and the cells can reduce nitrate to nitrite. Indole and H2S are not produced; however, hippurate is hydrolyzed. This organism is resistant to cephalothin but is sensitive to nalidixic acid. It has been isolated from the blood cultures of HIV-positive males with AIDS.

### Acknowledgments

We thank Graeme Stewart for permission to report clinical information relating to these patients; Peter Faby from the N.S.W. Department of Agriculture for performing GC on the isolates; and Adrian Lee, School of Microbiology and Immunology, University of New South Wales, for helpful discussions and review of the manuscript.

### References


