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Helicobacter sp. Strain *Mainz* Isolated from an AIDS Patient with Septic Arthritis: Case Report and Nonradioactive Analysis of 16S rRNA Sequence

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A campylobacter-like organism was isolated from an effusion of the left knee joint of an AIDS patient 2 weeks after bacteremia with a morphologically identical organism. Amplified genomic 16S rRNA sequences were analyzed by a nonradioactive blotting technique. The closest match was found with *Helicobacter fenelliae* (97.7% homology). Sequence data and phenotype suggest that the isolate may represent a so far unrecognized species of the genus *Helicobacter*.

A 35-year-old human immunodeficiency virus (HIV)-seropositive male with a 2-year history of AIDS (World Health Organization classification 3/3c) presented with fever and polyneuropathic symptoms in both legs at our clinic. Results from physical examination and chest X ray suggested pneumonia as the underlying cause of the fever. Cultures of sputum samples and blood taken at the time of admission did not result in the isolation of a pathogen during the 4 days of hospitalization. The patient was empirically treated with ofloxacin per os and was released from the hospital without signs of residual infection. Antibiotic therapy with ofloxacin was continued. Two weeks later the patient returned with a painful swelling that acutely affected his left knee joint. No other focal or systemic symptoms were evident. About 80 ml of a yellowish and turbid liquid was obtained from the joint.

Meanwhile, several aerobic blood cultures (BACTEC) taken at the time of the first admission had become positive (after 6 days of incubation and with growth indices of about 60). While Gram-stained smears of these cultures were negative, staining with acridine orange or Giemsa stain revealed a large number of curved and sometimes spiral organisms. Attempts to subculture this organism in aerobic or anaerobic BACTEC bottles or blood agar in either atmosphere failed. However, an organism with morphological and staining characteristics identical to those of the organism obtained at the first admission was then isolated from the effusion of the knee joint. Primary isolation and subcultures succeeded on blood agar under microaerophilic conditions at 37°C but not at 42°C. An identification system for anaerobic bacteria (rapid 32A; BioMerieux) failed. Biochemical tests for cytochrome oxidase and catalase were positive. No hydrolysis of indoxylacetate, no alkaline phosphatase activity, no reduction of nitrate, and no urease activity were detected. Biochemical tests were performed with diagnostic tablets from Rosco, Taastrup, Denmark (Indoxylacetate, alkaline phosphatase, and nitrate reduction) and with test kits from Difco (cytochrome oxidase and urease). Dark-field microscopic examination revealed motility reminiscent of *Campylobacter* species. Susceptibility to cephalotin (30 μg), tetracycline (30 IU), and clindamycin (2 IU) as well as resistance to nalidixic acid (30 μg), ciprofloxacin (5 μg), and erythromycin (15 IU) were evident on blood agar by a disc diffusion technique in which resistance was defined as no inhibition zone at all. The patient was treated with clindamycin for 2 weeks, and he fully recovered from arthritis.

Since we could not identify the presumed campylobacter-like organism by conventional microbiological methods, we decided to perform an analysis of amplified genomic 16S rRNA sequences. On the basis of those sequences, the phylogenetic distances between the organism in question and reference strains can be estimated (7, 19, 26). Total DNA was extracted essentially as described elsewhere (1), with the only modification being that the bacteria were taken directly from culture plates. Two different sequencing approaches based on digoxigenin-labelled sequencing primers were exercised: first, sequencing of single-stranded templates generated from cloned PCR products of 1.0 and 1.5 kbp and, second, direct solid-phase sequencing of the 1.5-kbp PCR product after single-strand template preparation by magnetic particle separation. Since essentially identical results were obtained, only the more convenient direct approach is described here. The primers used for amplification and sequencing correspond to highly conserved regions of the eubacterial 16S rRNA genes and have been described earlier (see reference 3 and the references therein). A fragment of 1.5 kbp was generated by PCR with primer pair pA-pH reverse. Two separate reactions with either of the two primers in a biotinylated version were performed. The mixtures contained 50 ng of total DNA in a total volume of 50 μl, deoxynucleoside triphosphates at a concentration of 1 mM each, primers at 0.1 μM, and 4 U of *Taq* polymerase (Boehringer-Mannheim, Mannheim, Federal Republic of Germany). The reaction buffer was supplied with the enzyme. Thermal cycling including an initial denaturation step of 5 min was done on a Triobloc instrument (Biometra, Göttingen, Federal Republic of Germany) by using the following cycling parameters: 36 cycles at 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s. Amplification was followed by a 10-min extension step at 72°C. Amplification products were analyzed on 1% agarose gels. The biotinylated strand of the PCR product was recovered with streptavidin-coated magnetic particles (Dynal, Oslo, Norway) according to the manufacturer's protocol. Particles were then used for direct nonradioactive solid-phase sequencing with the dideoxy-chain termination method with 5′-digoxigenin-labelled primers (oligonucleotides

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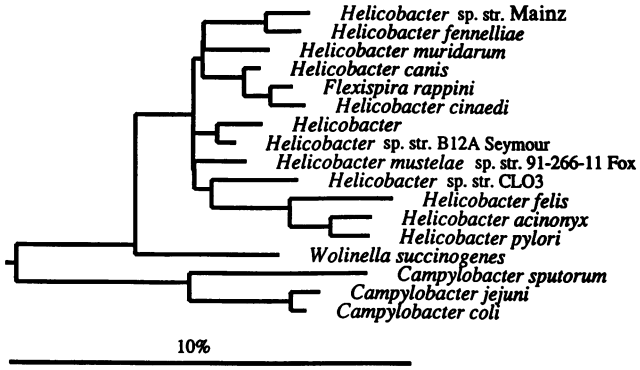

FIG. 1. Maximum likelihood tree reflecting the phylogenetic relationships of *Helicobacter* sp. strain *Mainz*, other members of the *Helicobacter* cluster, and selected reference organisms. The length bar indicates 10% estimated sequence divergence.

pA, pB, pC, pD, and pE with the noncoding strand and pC reverse, pD reverse, pE reverse, pF reverse, pG reverse, pH reverse, and pHel reverse for the coding strand). Primer pHel reverse (5′-GCTTCTCTTTGTGTGCCCCA-3′) is complementary to positions 1195 to 1214 of the coding strand of the isolate. Cycle sequencing proved to yield more readable sequence than classical sequencing with T7 polymerase and was done with the Dig-Taq cycle sequencing kit from Boehringer Mannheim. Sequencing reactions, separation on ultrathin, denaturing polyacrylamide gels, and simultaneous blotting onto nylon membranes with a GATC-1500 Direct Blotting Electrophoresis DNA Sequencer as well as detection by an alkaline phosphatase–Lumigen PD-based chemiluminescence reaction and visualization by autoradiography have been described earlier (9, 23), and all were done according to the manufacturers' protocols (MWG Biotech, Munich, Federal Republic of Germany, and Boehringer-Mannheim, respectively). Contiguous sequence information was obtained for 1438 bp. The new 16S rRNA sequence was added to an alignment of about 1,800 homologous primary structures from bacteria (12, 17) by using the alignment tool of the ARB program package (24a). Phylogenetic analyses were performed by applying distance matrix (ARB, PHYLIP [4]) maximum parsimony (ARB, PHYLIP), and maximum likelihood methods (fast DNAml [12]) on different data sets. The highest overall sequence similarity value (97.7%) was found for the pair *Helicobacter* sp. strain *Mainz* (named after the city where it was isolated) and *Helicobacter fennelliae*. The phylogenetic relationship of *Helicobacter* sp. strain *Mainz*, other representatives of the *Helicobacter* cluster, and selected reference organisms are shown in Fig. 1. The tree shown in Fig. 1 is based on the results of a maximum likelihood analysis of a subset of 16S rRNA sequences from bacteria of the S subclass of proteobacteria. Only alignment positions that share a common character in at least 50% of all available 16S rRNA sequences from δ-subclass proteobacteria were used for tree reconstruction. The topology of the tree was evaluated and corrected by applying different treeing methods on a variety of data sets. Multifurcations in the tree shown in Fig. 1 indicate branchings for which a relative order could not significantly be reconstructed or a common order was not supported by different analyses.

Identification of clinical *Helicobacter* isolates is generally achieved by evaluation of morphological, staining, and biochemical characteristics as well as antibiotic resistance patterns (2, 5). Protein patterns by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, antigenic profiles, analysis of fatty acid composition, DNA hybridization, and determination of the GC content have also been used in some instances (6, 11, 25). Phylogenetic analysis of 16S rRNA sequences was used to characterize the present strain and places it next to *H. fennelliae*. This species is associated with enteric disease in homosexual men (25). It has also been recovered from the blood of HIV-seropositive individuals (18). However, apart from exhibiting a significantly different 16S rRNA sequence, the isolate described here did not hydrolyze indoxylacetate, tested negative for alkaline phosphatase, and was resistant to nalidixic acid. Several cases of septic arthritis in HIV-seropositive individuals have been described previously (8, 10, 13–16, 20–22, 24). Among the isolated species were staphylococci, salmonella, mycobacteria, and nocardia and various fungi. We are not aware of another case involving *Helicobacter* spp.

Thus, clinical, phenotypic, and genotypic features distinguish *Helicobacter* sp. strain *Mainz* from other isolates. While we could not detect the source of infection in the patient described here, oligonucleotide probes derived from the 16S rRNA sequence might eventually lead to the discovery of the natural habitat of this strain. The strain has been deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Nucleotide sequence accession number. The almost complete 16S rRNA sequence is available from EMBL under accession number X81028.

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