**Helicobacter cinaedi** Cellulitis and Bacteremia in Immunocompetent Hosts after Orthopedic Surgery

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Received 21 July 2006/Returned for modification 11 September 2006/Accepted 18 October 2006

At various times after orthopedic operations (more than a few weeks, with an average of 29.9 days), 11 patients had a sudden onset of high temperature (average 38.9°C) and local cellulitis at different sites on the operated sides. The wounds had completely healed, without complicated infections, when the cellulitis occurred. The clinical picture of cellulitis in all patients was atypical: diffuse salmon-pink skin color, local heat, swelling, spontaneous pain, and tenderness but no eruptions. No patient had any underlying immunocompromising conditions or had been given immunosuppressive agents. Gram-negative spiral bacteria were isolated from blood cultures and were identified as *Helicobacter cinaedi* on the basis of 16S rRNA gene sequencing and DNA-DNA hybridization using standard strains. By means of phylogenetic analysis, we divided these clinical isolates into two clones. The *H. cinaedi* strain isolated via fecal cultures from two patients without intestinal symptoms was the same clone as the blood isolate. All isolates were quite susceptible to various antibiotics, and clinical and inflammatory symptoms of bacteremia and cellulitis improved after treatment with penicillins and cephalosporins. A relatively high incidence of recurrence of the same disease was observed, however. Almost all patients responded immunologically to the infection, as evidenced by the production of serum antibody against *H. cinaedi*. We thus suggest that *H. cinaedi* should not be regarded as simply an opportunistic pathogen but that it may be a pathogen in immunocompetent hosts and may cause infections together with bacteremia and cellulitis.

*Helicobacter cinaedi*, first identified as a *Campylobacter* species but now known to be an enterohelicate *Helicobacter* species, is usually found in the intestinal tract and/or liver of humans and other mammals (2, 8, 28, 30, 32, 35–37). The last two decades have seen increasing numbers of reports of *H. cinaedi* infections, mainly in humans and particularly in individuals with underlying immunosuppressive conditions such as AIDS, malignant diseases, and chronic alcoholism (3, 11, 18, 20, 23). Vandamme et al. previously documented a few cases of infection with *H. cinaedi* isolated from feces and blood from an apparently nonimmunocompromised child and adult (36).

Other groups have also described invasive infections caused by *H. cinaedi* and other *Campylobacter*-like organisms (*Helicobacter fennelliae*) in patients without underlying diseases (15, 26). However, despite these reports, the pathogenicity and etiological properties of this spiral bacterium are only poorly understood. Also, the molecular epidemiology of various strains isolated from humans has not yet been systematically analyzed.

We recently observed 11 cases of *H. cinaedi* bacteremia and cellulitis that occurred consecutively during a particular period in the same hospital. The clinical and epidemiological features of the *H. cinaedi* infections were investigated in the present study, which may thus provide important insights into the pathogenesis and epidemiology of this emerging pathogen.

**MATERIALS AND METHODS**

**Isolation and culture of bacteria from samples of blood and feces.** An aliquot of 10 ml of patients’ venous or arterial blood was cultured by using the BD BACTEC Plus Aerobic/F system according to the manufacturer’s protocols (Becton Dickinson and Company, Franklin Lakes, NJ). After the culture-positive notification, a part of the culture sample was inoculated onto Campylobacter agar (Becton Dickinson) and incubated at 37°C under microaerobic conditions (CampyPak microaerophilic system; Becton Dickinson) with high humidity. After Gram staining of the colony-forming bacteria, bacterial growth was examined microscopically. A fecal culture was performed similarly to the blood culture by inoculating and culturing the samples of feces on Campylobacter agar (Becton Dickinson). The spiral bacteria isolated were used for further investigation as described below.

**Identification of the species of the clinical isolates.** DNA from type strains of *H. cinaedi* (CCUG 18818) and *Helicobacter canis* (NCTC 12375) and representative strains of clinical isolates (isolate 377 from case 2 and isolate 717 from case 5) were prepared according to a standard procedure (21). DNA from each strain was labeled with photobiotin (Vector Laboratories, Inc., Burlingame, CA), and microplate qualitative DNA-DNA hybridization was performed according to previously described methods (6) to determine the taxonomic species name.

Phylogenetic analysis by using 16S rRNA and hsp60 gene sequences. 16S rRNA and hsp60 genes of all isolates were amplified by PCR as previously described (7, 16, 22). The sequences were determined by using an automatic sequencer (model 3100; Applied Biosystems, Foster City, CA) and dye terminator reaction kit (Applied Biosystems). About 1,430 bp of the 16S rRNA gene sequence and 530 bp of the hsp60 gene sequence were determined for each strain. To detect closely related species, each sequence discovered was analyzed by means of the FASTA search system (29) found at the DNA Databank of Japan (DDBJ) website (http://www.ddbj.nig.ac.jp). Sequences of the 16S rRNA and hsp60 genes of closely related species of the genus *Helicobacter* were taken from the DDBJ, GenBank, and European Molecular Biology Laboratory (EMBL) databases. CLUSTAL-X software, originally described by Thompson

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† Published ahead of print on 1 November 2006.
**H. cinaedi** infections

<table>
<thead>
<tr>
<th>Date of serum (yr)</th>
<th>Location of operation (cells/mm³)</th>
<th>Recurrence</th>
<th>Stool culture</th>
<th>Diarrhea</th>
<th>Antibiotic</th>
<th>Recurrence</th>
<th>Date of admission (Sort)</th>
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<td>6 58 M 717 12 R lateral thigh 14,830 19.3</td>
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**Analysis by RAPD.** Random amplified polymorphism DNA (RAPD) analysis was performed according to previously described methods (1, 4). After preliminary experiments, we selected two primers (primer 1281 [5'-AAC GGC CAA-3'] and primer 1283 [5'-GCC ATC CCC A-3']) for RAPD typing of the *H. cinaedi* strains. PCR was carried out in 50 µl of a mixture containing 1 µl of extracted DNA (~20 ng), 5 µl of 10X PCR buffer containing 20 mM MgCl₂, 2.5 µl of deoxynucleoside triphosphate mix (2.5 mM each), 5 µl of primer (4 µM), and 0.5 µl of hot-start EX-Taq (5 U/µl Takara Bio Inc., Shiga, Japan). The cycling program was 1 cycle each at 94°C for 2 min, 37°C for 1 min, and 72°C for 4 min and 29 cycles each at 94°C for 2 min, 37°C for 3 min, and 72°C for 7 min. After PCR, 10% agarose gel electrophoresis with ethidium bromide staining was performed, and the DNA fingerprint of each strain was evaluated.

**ELISA for determination of antibody response to the infection.** For the enzyme-linked immunosorbent assay (ELISA) for the determination of the antibody response to the infection, *H. cinaedi* whole-cell antigen was first prepared by sonication of bacterial cells of clinical isolates that had been cultured on and collected from Campylobacter agar according to a method reported previously (14). We first tested the antibody titer by use of whole-cell antigen prepared from several different clinical isolates (e.g., strains 923 and 1035 as representatives) (Table 1) of two different clones that we identified in the present genomic analysis. Because the ELISA showed almost similar responses for the sera from different patients, we used strain 1035 to prepare the antigen throughout the study. The whole-cell extract was then treated with Triton X114 to remove lipopolysaccharide as described previously (19), and a fraction obtained via ultracentrifugation (<100 kDa; Millipore, Billerica, MA) was used as an *H. cinaedi* antigen in the ELISA. Each well of a 96-well microtiter plate was coated with 50 µl of a mixture containing 1 µg of protein/well) in 0.1 M carbonate buffer (pH 9.6), blocked with 0.5% gelatin, and washed three times with phosphate-buffered saline containing 0.05% Tween 20 washing buffer. Samples (400-fold-diluted human serum) in the wells were incubated for 1 h at room temperature. Wells were then washed with washing buffer and reacted with horseradish peroxidase-conjugated anti-human immunoglobulin G antibody (Sigma-Aldrich Corporation, St. Louis, MO), followed by a reaction with 1,2-phenylenediamine dihydrochloride. The reaction was terminated by the addition of 50 µl of 2 mol/liter sulfuric acid, and absorbance at 490 nm was read by means of a micro-ELISA plate reader. To examine the host immune response against *H. cinaedi* infection, serum levels of antibody against *H. cinaedi* antigen and *H. cinaedi*-infected groups and those of three different control groups, *Heliocobacter pylori*-infected subjects, age- and sex-matched controls without apparent *H. cinaedi* infection, and infant subjects younger than 1 year, were compared.

**Nucleotide sequence accession numbers.** All sequence data determined in this work were deposited in the DDBJ/GenBank/EMBL databases under the accession numbers listed in Fig. 2.

**RESULTS**

**Case reports.** During 9 months from July 2003 to March 2004, 11 patients who had been admitted to Kumamoto Orthopedic Hospital (204 beds) in Kumamoto, Japan, for orthopedic surgery suffered from bacteremia and cellulitis caused by *H. cinaedi*. Table 1 summarizes the clinical characteristics of...
the patients (two men and nine women). These patients, whose ages ranged from 22 to 79 years (average, 65.5 years), had a sudden onset of cellulitis accompanied by high temperature. The clinical symptoms appeared an average of 29.9 days (range, 8 to 113 days) after the orthopedic operations. At the time that the patients showed these symptoms, wounds at operative sites had completely healed. *H. cinaedi* was isolated from cultures of blood obtained from all patients: four isolates from venous blood and seven isolates from arterial blood. In the past, three patients (79-, 76-, and 65-year-old women) had had hypertension, but it was well controlled when they were infected, and patients’ histories contained no records of autoimmune or malignant diseases or their treatment. Also, the patients did not have any underlying disorders that would cause an impairment of antimicrobial host defense, nor did they have abnormal findings from physical and laboratory examinations except for the orthopedic diseases, i.e., bone fractures and osteoarthritis (Table 1), which necessitated the orthopedic surgeries. Operations included six operations for bone fractures (a 22-year-old man had an open fracture), four for artificial knee joint replacement for osteoarthritis, and one for lumbar herniation. All patients had cellulitis on their operated side together with fever (38.5°C to 39.5°C; highest mean temperature, 38.9°C); at that time, *H. cinaedi* organisms were isolated from blood culture. One patient (case 1) had a symptom of enteritis (diarrhea); however, *H. cinaedi* was not isolated from this patient by fecal culture. *H. cinaedi* organisms were isolated from two other patients without intestinal symptoms via fecal cultures (cases 9 and 11). Two patients (cases 5 and 7) underwent two operations at different times, and each time, cellulitis occurred in the operated side, with isolation of bacteria from blood culture.

All patients showed atypical clinical symptoms of cellulitis, including diffuse, pale, salmon-pink skin color; local heat; swelling; spontaneous pain; tenderness; and no eruptions. Figure 1 provides a representative picture of the appearance and magnetic resonance image of cellulitis. Cellulitis occurred in the left lower leg, the same side as the operation but not near the surgical incision and wound, 42 days after the surgery. The lesion of this patient was accompanied by swelling, salmon-pink skin color, local heat, spontaneous pain, and tenderness. Coronal and axial T2-weighted magnetic resonance images of the left lower leg confirmed subcutaneous edematous and inflammatory changes, which are typical findings of cellulites. In all cases, healing of the surgical wounds had been completed by the time of the onset of cellulitis, and there were no apparent infection-related findings such as redness, pain, swelling, and purulent discharge in the wound tissues. Hematology and blood chemistry studies indicated leukocytosis and elevated C-reactive protein and erythrocyte sedimentation rate values when patients had cellulitis and fever. All these symptoms of inflammation were thought to be unrelated to a direct effect of the operations, because they appeared more than 1 week after the date of surgery, when the surgical wounds had already completely healed. The clinical symptoms appeared an average of 29.9 days (range, 8 to 113 days) after the orthopedic operations. At the time that the patients showed these symptoms, wounds at operative sites had completely healed. *H. cinaedi* was isolated from cultures of blood obtained from all patients: four isolates from venous blood and seven isolates from arterial blood. In the past, three patients (79-, 76-, and 65-year-old women) had had hypertension, but it was well controlled when they were infected, and patients’ histories contained no records of autoimmune or malignant diseases or their treatment. Also, the patients did not have any underlying disorders that would cause an impairment of antimicrobial host defense, nor did they have abnormal findings from physical and laboratory examinations except for the orthopedic diseases, i.e., bone fractures and osteoarthritis (Table 1), which necessitated the orthopedic surgeries. Operations included six operations for bone fractures (a 22-year-old man had an open fracture), four for artificial knee joint replacement for osteoarthritis, and one for lumbar herniation. All patients had cellulitis on their operated side together with fever (38.5°C to 39.5°C; highest mean temperature, 38.9°C); at that time, *H. cinaedi* organisms were isolated from blood culture. One patient (case 1) had a symptom of enteritis (diarrhea); however, *H. cinaedi* was not isolated from this patient by fecal culture. *H. cinaedi* organisms were isolated from two other patients without intestinal symptoms via fecal cultures (cases 9 and 11). Two patients (cases 5 and 7) underwent two operations at different times, and each time, cellulitis occurred in the operated side, with isolation of bacteria from blood culture.

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healed. Antibiotic chemotherapy was effective, so cellulitis and fever along with other inflammatory findings improved within a few days after starting treatment. However, 4 of 11 patients had recurrences of cellulitis in the same area, although the degree of severity of the cellulitis diminished gradually with each recurrence. For example, one patient (case 6, a 58-year-old man) had five recurrences of cellulitis during follow-up at an outpatient clinic, and for the later recurrences, the cellulitis spontaneously disappeared without any specific treatment.

Culture and isolation of bacteria. Culture of blood from all 11 patients via the BACTEC Plus system produced positive results after 4 to 7 days of incubation of cultures. Microscopic examination of bacterial organisms grown in this system revealed that all strains were a gram-negative spiral-shaped bacterium. Subculture from the culture bottle on Campylobacter agar resulted in growth under microaerobic conditions, but the organisms were very fastidious with regard to growth conditions such as nutrient and gas composition and humidity. For example, on a Columbia blood agar plate (bioMérieux, Marcy l’Etoile, France) or other media under the same culture conditions, isolates manifested no growth. A CO2 incubator for tissue cultures (supplied with 5% CO2 gas) with humidification or an anaerobic incubator (N2 base gas with 10% CO2 and 10% H2) also resulted in no effective growth of this organism. The fecal culture for the two patients who had no apparent enteritis symptoms grew the same gram-negative spiral bacterial organisms as those grown via blood culture.

Identification of bacterial strains. All isolates showed very high 16S rRNA gene sequence similarity to the type strains of H. cinaedi (more than 99.3%), whereas less than 98.5% similarity to other species of the genus Helicobacter was found (Fig. 2). The genomic DNA-DNA hybridization result revealed that the isolates (two representative strains) belonged to the species H. cinaedi, because they showed more than 82% homology with the type strain of H. cinaedi (Table 2).

Epidemiological features of the isolates. Molecular epidemiology of the clinical isolates was studied by means of phylogenetic analysis. On the phylogenetic tree, the type strain of H. cinaedi and all isolates formed one cluster (Fig. 2) that comprised two subclusters, the first composed of four strains, which were isolated at the time of the initial H. cinaedi outbreak (cases 1 to 4), and the second containing the other six isolates from the later outbreak (cases 5 and 7 to 11). The type strain of H. cinaedi was located at a position intermediate between the subclusters. The DNA sequences of strains within each subgroup were identical. Five and nine base substitutions were found for the type strain versus isolates of the first subcluster and the second subcluster, respectively; 12 base substitutions were found between the isolates of the first and second subclusters.

![FIG. 2. Genetic analysis of clinical isolates of H. cinaedi. 16S rRNA (A) and hsp60 (B) genes were analyzed by means of the FASTA search system. Phylogenetic relationships for these clinical isolates and representative members of the genus Helicobacter were analyzed on the basis of the 16S rRNA gene sequence (1,430-bp area) and hsp60 gene sequence (530-bp area) obtained from the DDBJ, GenBank, and EMBL databases. The accession numbers for the 16S rRNA and hsp60 gene sequences of each strain are shown in parentheses.](http://jcm.asm.org/)
Almost the same result was observed with hsp60 gene sequence analysis (Fig. 2). All isolates and the type strain of \( \text{H. cinaedi} \) clearly made one cluster, with two subclusters appearing within it. Members of each subcluster were exactly the same as those found via 16S rRNA gene phylogenetic analysis.

As shown in Fig. 3, PFGE revealed that the type strain of \( \text{H. cinaedi} \) and clinical strains were completely different: some large bands (more than 112.0 kb) were missing, and some extra intermediate-sized bands (48.5 to 112.0 kb) existed only in the type strain. Also, four isolates from the initial infections (cases 1 to 4) and four from the later outbreak (cases 5, 7, 8, and 9) showed slightly different PFGE patterns. RAPD analysis provided similarly different profiles for the genome of the \( \text{H. cinaedi} \) isolates (Fig. 4).

**DISCUSSION**

Here, we describe 11 cases of \( \text{H. cinaedi} \) bacteremia associated with cellulitis but without apparent immunocompromised conditions, particularly human immunodeficiency virus infection. Previously published epidemiological studies and case reports indicated a strong association of the onset of \( \text{H. cinaedi} \) infection with an immunocompromised state and/or human immunodeficiency virus infection or a specific condition, e.g., homosexuality (3, 5, 8, 11, 24, 31, 32). We believe, however, that the cases of \( \text{H. cinaedi} \) infections that we detail here all occurred in apparently immunocompetent subjects, although they may have been prone to develop \( \text{H. cinaedi} \) infections, as discussed below.

One unique feature of the present \( \text{H. cinaedi} \) infections is
that these 11 cases occurred as a cluster at the same hospital, all after orthopedic operations. These cases may thus represent a nosocomial outbreak. Epidemiological and phylogenetic analysis showed that the clinical isolates could be classified into two groups with several base substitutions. One group of \textit{H. cinaedi} isolates was obtained from patients who stayed in the hospital ward, mainly on the seventh floor, from June to September 2004, and the other group was derived from patients who stayed on the sixth floor of the same ward from October 2004 to March 2005 (Table 1). Therefore, each clone seems to have spread in a relatively limited environment in the hospital and in a serial fashion during several months.

Because of this epidemiological evidence and because all cases occurred after orthopedic surgery, the present outbreak may have an iatrogenic cause or may have originated from physical areas and people (other patients and hospital staff) that were contaminated with \textit{H. cinaedi} organisms. A prospective surveillance study was therefore initiated to clarify the epidemiology of \textit{H. cinaedi}, and infection control measures were reinforced according to recommendations of the Hospital Infection Control Practice Advisory Committee. Because \textit{H. cinaedi} is thought to have an oral route of infection, water from a feed tank was cultured for the presence of bacteria. Also, swab cultures of surgical instruments and materials used for nursing care of patients in hospital rooms, e.g., wet towels, were taken. More importantly, to identify any \textit{H. cinaedi} carriers in the hospital, stool samples for culture were obtained from patients without any symptoms of \textit{H. cinaedi} infection and medical staff members, including physicians, nurses, and physiotherapists, who had close contact with the infected patients. However, all these surveillance measures produced negative results for the presence of \textit{H. cinaedi} organisms.

Therefore, although the apparent source and route of this outbreak remain unclear, because \textit{H. cinaedi} was isolated via fecal culture from 2 of 11 patients, the \textit{H. cinaedi}-infected patients themselves may be the source of the outbreak, which may have spread via an oral route. We also found that one physiotherapist who had no clinical symptoms and negative fecal culture results showed a high serum antibody titer for \textit{H. cinaedi} organisms. A prospective study was therefore initiated to clarify the epidemiology of \textit{H. cinaedi}, and infection control measures were reinforced according to recommendations of the Hospital Infection Control Practice Advisory Committee. Because \textit{H. cinaedi} is thought to have an oral route of infection, water from a feed tank was cultured for the presence of bacteria. Also, swab cultures of surgical instruments and materials used for nursing care of patients in hospital rooms, e.g., wet towels, were taken. More importantly, to identify any \textit{H. cinaedi} carriers in the hospital, stool samples for culture were obtained from patients without any symptoms of \textit{H. cinaedi} infection and medical staff members, including physicians, nurses, and physiotherapists, who had close contact with the infected patients. However, all these surveillance measures produced negative results for the presence of \textit{H. cinaedi} organisms.

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been replaced by the other clone during the outbreak period. The mechanism of substitution of one clone for the other is unclear. It may have resulted from a genetic mutation that occurred during the spread of bacteria, or two coexisting clones may have infected and/or colonized a particular individual or the hospital or patient community environment. Also, because the same clone was recovered from both blood and fecal samples of a few patients during the outbreak, *H. cinaedi* organisms might be transmitted via an oral route among human subjects, colonize the intestinal tract, and then, under particular conditions such as orthopedic surgery, disseminate via the vascular system to cause bacteremia and cellulitis.

In conclusion, we report here a unique outbreak of *H. cinaedi* infections caused by two different bacterial clones. Although the epidemiology of the infection is not fully understood, these patients did not have the particular risk factors or underlying conditions, except for prior surgical operation, that might lead to this infection. Because of the lack of highly sensitive bacterial identification techniques, other immunocompetent subjects infected with *H. cinaedi* may have been overlooked in the past. Our present findings now warrant further investigation of the pathogenesis, etiology, and epidemiology of this emerging disease.

ACKNOWLEDGMENTS

We are very grateful to Judith B. Gandy for her excellent editing of the manuscript. We thank Tatsuya Kawaguchi, Chief of Infection Control Team at Kumamoto University Hospital, and Tadashi Nakamura and Shuichi Higashi at Kumamoto Orthopedic Hospital and Keita Yamakawa, a former member of the Infection Control Team at the same hospital, for their helpful discussion and cooperation in the surveillance study. Thanks are also due to Akira Nishizono, Oita University, for providing us with serum from patients infected with *H. pylori* and neonate serum and with technical support for the ELISA.

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, and Science (MEXT) and by a grant for the future science promotion program from Kumamoto University.

REFERENCES


