The Nosocomial Transmission of *Helicobacter cinaedi* Infections in Immunocompromised Patients

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Abstract

**Background** We encountered 15 cases of *Helicobacter cinaedi* (*H. cinaedi*) infection between March and July 2008.

**Patient, Method, and Result** The underlying diseases were hematological malignancies in a majority of cases, many of which received chemotherapy. All patients had a fever. The fever was followed by cellulitis in three, a skin rash in six, pain in the lower limbs in three, and diarrhea in three cases. We analyzed the bacterial 23S rRNA genes. The fifteen strains were divided according to base sequence into Groups A, B, and C, respectively. All four cases in Group A were women and all ten in Group C were men, indicating that the gender of the patient corresponded precisely to the genotypes of the separated bacilli in these two groups. These findings also suggested the strong possibility of nosocomial spread.

**Conclusion** It is highly likely that *H. cinaedi* infections have been overlooked due to the difficulties encountered in culturing the bacterium. The possibility of septicemia caused by *H. cinaedi* should be suspected especially in immunocompromised patients such as those undergoing chemotherapy, with symptoms such as fever, rash, arthritis, cellulitis, leg pain, and other systemic or local symptoms.

**Key words:** *Helicobacter cinaedi*, nosocomial transmission, bacterenemia, cellulitis, immunocompromised host, febrile neutropenia

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**Introduction**

*Helicobacter cinaedi* (*H. cinaedi*) is a Gram-negative spiral bacillus that lives in the intestinal tracts of various animals, including humans, and has a wide range of reservoirs. It is known to cause diarrhea as well as bacteremia, cellulitis, and other clinical symptoms after entering the body through the intestinal mucosa (1-5). Although such symptoms were observed primarily among HIV-infected patients (4, 5), their occurrence among other immunocompromised patients, and those with renal or hepatic diseases (4, 6, 7) were reported recently. Furthermore, infections among immunocompetent patients such as cases of infection in an orthopedic ward (1) and a case of mother-to-child transmission have been reported (8). These instances suggest the possibility that *H. cinaedi* exists widely in nature but has been overlooked.

As we encountered a series of 15 *H. cinaedi* infection cases in our ward, we studied these cases: 1) to clarify the clinical presentation of *H. cinaedi* infection by examining the patient background, underlying disease, clinical symptoms, treatment, and other details of these 15 cases in our department, and 2) to determine the infectious route through extensive bacteriological and genetic testing. As a result, these cases were strongly suspected of being nosocomial in origin because they occurred consecutively in the same ward within a short period and the clinical symptoms varied in each instance.
Patients and Methods

We examined 15 patients who developed *H. cinaedi* infections in the Rheumatology and Hematology Ward of our hospital between March and July 2008. The *H. cinaedi* infections were defined as those with fever, cellulitis, skin rash, and diarrhea along with other clinical symptoms, as well as those where *H. cinaedi* was detected in blood or stool cultures.

Blood culture was performed using an automatic blood culture system (BACTEC9120, Nippon Becton Dickinson Co., Ltd., Japan) with 92F aerobic and 93F anaerobic resin bottles. Because the median time until the detection of *H. cinaedi* is at least six days (9), which is longer when compared with that of more common bacteria, the culture period was set as ten days. For stool culture, microaerobic culture at 35°C was conducted using modified Skirrow agar EX (Nissui Pharmaceutical, Japan) and a culture period of seven days rather than usual two days due to the longer period required for the detection of *H. cinaedi*.

The strains that formed distinctive film-like colonies on the plate and were shown to be Gram-negative with a spiral shape; they were further identified as *H. cinaedi* by genetic testing. For the genetic tests, polymerase chain reaction (PCR) was performed in accordance with the method presented by Ohkusu et al (9), using TATACCGGGTAAGGAGT GCTGGAG and ATCAATTAACCTTCGAGCACCG as primers to amplify the 23S rRNA gene region common to all types of *Helicobacter* bacteria. We also used the AGGG ATTTCCAAGATGAGC and TCTTGTCTGTGCGTTCA TC primer sequences to amplify the gyrB gene region that is specific to only *H. cinaedi*. We identified the samples having both genes amplified as *H. cinaedi*. We conducted genetic typing by amplifying some of the 23S rRNA genes by PCR and used these sequences to conduct interstrain comparisons. For these PCR experiments, we used the forward primer CGATAAGCTATGGGGAGCT and reverse primer A TCAATTAACCTTCGAGCACCG. The resulting 1.8-kbp PCR products were compared by determining the base sequences in both directions using the same primer set and three other primers for sequences (ACCCGACTAACCCTAC GA, TTTGACAGGGTTGGTAC, and TATACCGGTAAAGT GCTGGAG).

Results

Case reports

Case 4

A 65-year-old man with malignant lymphoma, who was receiving chemotherapy (R-CHOP), presented with a fever of 38°C on Day 9 of the first course of R-CHOP. He was diagnosed as febrile neutropenia (FN) because the focus of the infection was unknown and accompanied with neutropenia, and the fever was relieved immediately after administration of antibiotics. Fever recurred on Day 10 of the second course, and a swelling of the right forearm with tenderness and burning sensation was observed (Fig. 1). As cellulitis was diagnosed, administration of antibiotics was commenced and the condition improved. Since *H. cinaedi* was detected by the blood culture, it was suggested as the cause of cellulitis. His fever recurred on Day 6 of the third course, and *H. cinaedi* was detected in the blood culture; his condition improved following antibiotic administration.

Although these treatments were only temporarily effective, the symptoms did not become severe and were considered extraneous to the vital prognosis. The administration of antibiotic agents was stopped and only supportive care was taken. Consequently, five months after the cessation of his chemotherapy, improvements in antipyretic tendency, redness, and swelling were observed. His overall condition improved by the sixth month.

Case 13

A 67-year-old woman was receiving immunosuppressive therapy for very severe-type aplastic anemia (VSAA). Fever and erythema, primarily on the chest and back, were observed three weeks after the treatment was started (Fig. 2). It seemed to be a reactive rash because no burning sensation or swelling was observed. Fever broke and the erythema disappeared immediately after administration of antibiotics. She was diagnosed with a reactive rash caused by *H. cinaedi*, which was detected in a stool culture performed in the same period. No recurrence of *H. cinaedi* infection has been observed.

Patients results

Table 1 summarizes the clinical and laboratory features and treatment responses of each patient. Of the fifteen patients in our study, ten were men and five were women, with a median age of 70 years. The majority of cases were hematological malignancies, many of which received chemotherapy. Immunosuppressive therapy was conducted for the other three cases. All fifteen patients had a fever. The fever was followed by cellulitis in three, a skin rash in six, pain in the lower limbs in three, and diarrhea in three cases. Only three patients suffered from multiple symptoms con-
current with fever.

Of the fifteen cases, *H. cinaedi* was detected in blood cultures of nine cases and stool cultures of eight subjects. The median time between the start of blood culture and the actual detection of the bacterium was five days (3-6 days), which was the upper limit of the standard culture period.

On laboratory tests, the mean values of WBC and CRP were 4,733/μL (600-24,000/μL) and 5.57 mg/dL (0.13-22.23 mg/dL). No tendencies were observed with regard to inflammatory markers, partially due to the influence of the treatments. However, the absolute CD4 count, which is an immunological indicator, was 304.25/μL (71-545/μL) in 8 of 15 patients within one month before detection of *H. cinaedi*, which is lower than the 400/μL level at which opportunistic infections are thought to be likely. Similarly, IgG levels were observed to be 863 mg/dL (617-1,352 mg/dL), which were lower than the normal range.

An initial therapy was provided using amoxicillin (AMPC), cefepime (CFPM), imipenem cilastatin (IPM/CS), sulbactam ampicilline (SBT/ABPC), and other penicillin, cephem, carbapenem, and quinolone drugs. Outside of one unassessable case, immediate symptomatic improvements were observed in twelve of the fifteen cases. The other two cases were refractory, and the symptoms persisted for three to six months despite the use of antibiotics. Although exacerbation was observed in six cases, they all responded quickly to re-treatment.

**Genetic results**

The fifteen bacterial strains that were confirmed as *H. cinaedi* using PCR analysis (Fig. 3) were further analyzed by gene typing, which typically employs pulse-field electrophoresis and other means to compare DNA banding patterns. However, because *H. cinaedi* often shows similar patterns even if the strains are not genetically related, the base sequences of some of the 23S rRNA genes were analyzed and compared in our study. It was found that, of the approximately 1.8-kbp products for which base sequences were determined, bases 44, 145, 647, and 1,497 differed by strain. All remaining sequences were identical. On the basis of these base patterns, the fifteen strains were divided into three groups (Table 2). Using the order of base sequences (44, 145, 647, and 1,497), groups showing G-T-T-G, A-T-T-A, and G-C-C-G were categorized as Groups A, B, and C, respectively. All four cases in Group A were women (Cases 1, 3, 5, and 13) and all ten in Group C were men (Cases 2, 4, 6, 8-12, 14, and 15), indicating that the gender of the patient corresponded precisely to the genotypes of the separated bacilli in these two groups. Group B included only one woman. It was highly likely that Case 1 in Group A and Case 2 in Group C were the probands; however, anthropozoososis was also suspected for Case 1 because that patient had a pet dog.

For the *H. cinaedi* infection that occurred in multiple patients, strains of the same genotypes were found in different patients. Therefore, the possibility of nosocomial spread was strongly indicated. Despite the fact that environmental surveillance revealed no definite evidence of the existence of *H. cinaedi* in our ward, we highly suspected that the infection spread via toilets for the following reasons:1) No patient staying in a private room was affected with *H. cinaedi* infection. 2) The bacilli lives in the intestinal tract and, ex-
except for one case, the genotypes of the separated strains corresponded completely with the gender of the patients. On the basis of these reasons, the cohorting of patients was introduced in addition to isolating patients in private rooms with toilets and the use of designated toilets. In addition, hand-washing and other instructions were given to everyone involved with the patients in order to stop the spread of the organism. The group infection was subsequently cleared.

### Table 1-2.

<table>
<thead>
<tr>
<th>Case</th>
<th>WBC (×10³/µL)</th>
<th>Neut (%)</th>
<th>Lym (%)</th>
<th>CRP (mg/dL)</th>
<th>Other isolated bacterium (Stool culture)</th>
<th>Initial Treatment (days)</th>
<th>Outcome</th>
<th>Recurrence</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>6800</td>
<td>91</td>
<td>7</td>
<td>3.38</td>
<td>CFP(4), AMK(4)</td>
<td>improvement</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6300</td>
<td>91</td>
<td>8</td>
<td>22.23</td>
<td>IPM/CS (14)</td>
<td>improvement</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>89</td>
<td>9</td>
<td>7.36</td>
<td>AMPC (14)</td>
<td>improvement</td>
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<td></td>
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<tr>
<td>4</td>
<td>400</td>
<td>N.E.</td>
<td>N.E.</td>
<td>8.49</td>
<td>E.coli, Enterococcus sp</td>
<td>CAZ (7), AMPC (28)</td>
<td>NR</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>24000</td>
<td>96</td>
<td>3</td>
<td>0.13</td>
<td>E.coli, Enterococcus sp</td>
<td>CFP(4), AMK(4), AMPC (28)</td>
<td>NR</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>1700</td>
<td>29</td>
<td>30</td>
<td>0.2</td>
<td>SBT/ABPC (7)</td>
<td>improvement</td>
<td>N.E.</td>
<td></td>
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<tr>
<td>7</td>
<td>600</td>
<td>18</td>
<td>74</td>
<td>0.27</td>
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<tr>
<td>8</td>
<td>2000</td>
<td>77</td>
<td>21</td>
<td>13.12</td>
<td>CFP(7)</td>
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<tr>
<td>9</td>
<td>11700</td>
<td>84</td>
<td>12</td>
<td>4.92</td>
<td>AMPC (14)</td>
<td>improvement</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2100</td>
<td>96</td>
<td>2</td>
<td>13.95</td>
<td>Kleb. Oxytoca, Enterococcus sp</td>
<td>CFP(8)</td>
<td>improvement</td>
<td>Yes</td>
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<tr>
<td>11</td>
<td>5900</td>
<td>94</td>
<td>5</td>
<td>1.83</td>
<td>CFP(7), AMK (2)</td>
<td>improvement</td>
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<td></td>
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<tr>
<td>12</td>
<td>1600</td>
<td>54</td>
<td>40</td>
<td>6.48</td>
<td>E. coli, Enterococcus sp</td>
<td>SBT/ABPC (7)</td>
<td>N.E.*</td>
<td>N.E.</td>
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<tr>
<td>13</td>
<td>600</td>
<td>21</td>
<td>72</td>
<td>0.71</td>
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<td>MEP(7)</td>
<td>improvement</td>
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<tr>
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<td>82</td>
<td>7</td>
<td>0.55</td>
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<td>CFP(7)</td>
<td>improvement</td>
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<tr>
<td>15</td>
<td>4800</td>
<td>58</td>
<td>32</td>
<td>0.19</td>
<td>CFP(14), AMK (14)</td>
<td>improvement</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>


### Discussion

A culture of *H. cinaedi* was first isolated in 1984 by Fennel et al from a homosexual man who showed colitis symptoms (10). It was first reported as a campylobacter-like organism. After that it was categorized as *Helicobacter* since the genus was created in 1989 (9).

*H. cinaedi* infections are confirmed by identifying the bacillus in blood or stool cultures; however, the identification of the organism from blood or stool is just as difficult as it
Figure 3. PCR amplification of 23S rRNA common to all types of *Helicobacter* bacteria.

**Table 2.** *H. cinaedi* Strains are Divided Into Three Groups. Using the Order of Base Sequences (44, 145, 647, and 1,497), Groups Showing G-T-T-G, A-T-T-A, and G-C-C-G were Categorized as Group A, B, and C

<table>
<thead>
<tr>
<th>Group</th>
<th>Case No</th>
<th>Male</th>
<th>Female</th>
<th>base No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1, 3, 5, 13</td>
<td>0</td>
<td>4</td>
<td>G T T G</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>A T T A</td>
</tr>
<tr>
<td>C</td>
<td>2, 4, 6, 8, 9, 10, 11, 12, 14, 15</td>
<td>10</td>
<td>0</td>
<td>G C C G</td>
</tr>
</tbody>
</table>

is for other *Helicobacter* species except for *H. pylori* (3). We believe that the following points are responsible. First, *H. cinaedi* requires a long culture period. It takes six days on average and up to ten days in some cases to detect in blood culture bottles (11), compared with the usual two to three days for *Campylobacter*. Second, the bacillus is unlikely to propagate in a carbon dioxide environment that is conducive to the growth of common bacterial strains or in microaerobic cultures that allow for the separation of *Campylobacter*.

The blood culture period is set at five days in many facilities, and the temperature of Skirrow and other culture media is set at 42°C for selective separation of *Campylobacter jejuni* from ordinary stool cultures. However, considering that the optimum culture temperature of *H. cinaedi* is between from 35 to 37°C, it is presumed that *H. cinaedi* infection is tend to be overlooked in many cases. The median time until detection in blood culture at our hospital was five days (three to six days), and this was relatively short when compared with previous reports. Because the *H. cinaedi* infections occurred consecutively in the same period, its transmission was strongly suspected, and it was presumed that the detection rate could be improved by setting the blood and stool culture conditions to suit *H. cinaedi*. A method for detecting *H. cinaedi* by targeting a membrane protein unique to the bacillus has also been presented (12), and it would be desirable to generalize its use in the clinical setting.

Even though *H. cinaedi* lives in the intestinal tract, many studies have shown that its clinical symptoms include fever, rash, arthritis, cellulitis, leg pain, and other systemic or local symptoms rather than abdominal pain, diarrhea, and other digestive symptoms. It is interesting that in our current study, only three cases out of fifteen presented digestive symptoms, and only one out of the eight where *H. cinaedi* was detected in stool culture had diarrhea.

Cytolethal distending toxin (Cdt) inhibits the cell cycle by stopping it in the G2/M phase, causing cellular distension for 24 to 48 hours, and leading to cell death in 96 to 120 hours (13). Taylor et al (14) reported that the production of Cdt by *H. cinaedi* can cause cytotoxicity. Although *H. cinaedi* lives in the intestinal tract and forms bacterial flora (11), it is possible that flagellar movement aids the adherence of *H. cinaedi* to the mucosal epithelium of the host. The localized production of Cdt may then facilitate the entry of the bacterium into the bloodstream by damaging the epithelial cells. The consequent systemic distribution of the organism may then cause bacteremia and other symptoms related to *H. cinaedi* infections.

Epithelial cells are known to have a short cell cycle, and this may be related to the frequent occurrence of rash, cellulitis, and other skin symptoms in the cases of *H. cinaedi*.
infection. These skin symptoms and pain are mainly observed in the limbs. Concerning this point, Kitamura et al (1) assumed the possibility that inadequate blood flow occurs locally because of cold stimulation, which leads to the entry of H. cinaedi into tissues and causes the clinical symptoms. Based on this assumption, they reported that turning off air conditioners in sites affected by these infections allowed them to be contained (1). In the present study, all cases of cellulitis developed in the limbs of patients, and our results were similar with those of past studies. It may be effective to keep the limbs warm in cases with recurring cellulitis.

Rashes spread mainly on the trunk of the patients in the present study and were improved soon after the commencement of treatment, indicating an obvious difference in the clinical presentation of cellulitis caused by H. cinaedi. Considering the associated presence of arthralgia, this may be an allergic reaction to a bacterial compound or a certain protein produced by H. cinaedi. Further studies will be necessary concerning this point.

In the present study, there was a case in which fever, rash, and other clinical conditions recurred repeatedly during the early stages of chemotherapy (Case 8). Nishine et al also reported a case where H. cinaedi septicemia developed repeatedly in a lung cancer patient soon after the commencement of chemotherapy (7). Febrile neutropenia is defined as infection without any obvious focus, occurring concurrently with the neutropenic phase. Considering that 1) this bacilli is common in nature and can form bacterial floras in the human intestinal tract, 2) mucosal damage often occurs due to chemotherapy and may lead to the repeated entry of H. cinaedi into the blood stream, and 3) the response to antimicrobial agents is relatively good, some of the disorders that were diagnosed as FN may actually be septicemia caused by H. cinaedi. It will be necessary to study the data of cases collected at various facilities to verify this hypothesis.

No guidelines have been established on the use of antibiotics against H. cinaedi. Penicillin, carbapenem, aminoglycoside, and quinolone were used and their effectiveness has been reported. However, the presence of erythromycin-resistant strains (15) and also repeated exacerbations following quinolone administration (6) have also been reported. Exacerbations were observed in eight out of the fourteen assessable cases in the present study. However, because the response to repeated administration of antibiotics was fast even after the exacerbations in many cases, those exacerbations were thought to be due to reasons other than drug resistance, as in past studies (1). Although infections caused by H. cinaedi recurred repeatedly in Cases 6 and 12 and the long-term administration of antibiotics was required, the conditions improved naturally about six months after the completion of chemotherapy.

The routes of H. cinaedi infection have not been clarified. Because this bacillus basically lives in and has been separated from the intestinal tract of humans and various other animals (9) (e.g., rat, hamster, dog, cat, fox, etc), possible infection routes may include contact with carrier animals and exposure to contaminated water. In the present study, four of thirteen patients had pets (Cases 1, 3, 5, and 10), and the possibility of the animal—human transmissions cannot be ruled out.

There have been several studies reporting nosocomial infection caused by H. cinaedi (1). A study by Kitamura et al documenting a group infection of H. cinaedi in an orthopedic ward (1) identified two different strains of H. cinaedi using PCR, although the infection occurred in multiple patients in the same ward in the same period. Despite environmental surveillance, in which culture was performed from surgical instruments, water tanks, nursing care instruments, wet towels, and various other items, H. cinaedi was not detected on any of these items and the route of the infection was not identified (1).

We divided thirteen strains into three genotypes in our study. It would be possible to consider that these three types of clones were originally the same clone, and the genetic mutations occurred while it had spread (1). Alternatively, these three strains may have been original and transmitted to different patients through nosocomial infection. It is also possible that these thirteen strains may have been completely different clones, each causing an infection in a separate patient during the same period purely by coincidence. However, because the genotypes of the separated strains corresponded with the gender of the patients in this study, we suspect that it was highly likely that these three strains were originally present, were transmitted separately, and were spread to multiple patients through toilets and other media.

In past studies, H. cinaedi was reported to be transmitted from household pets. However, it may also be considered that this bacillus can also be transmitted between individuals and may cause nosocomial infection. This concept seems to be supported by our results and the fact that the outbreak ended once infection control measures, such as the use of designated toilets, isolation of patients, and thorough hand-washing practices among patients and medical staff, were implemented. It is also presumed that a considerable number of carriers exist in the population, considering that some infected individuals do not develop clinical symptoms (1) and that it is highly likely that the development of infection by this bacillus depends on the immune status of each patient.

Although there have been no cases of death associated with H. cinaedi infections, the bacterium was difficult to eliminate as indicated by its recurrence immediately after the discontinuation of treatment. This fact considerably lowered the quality of life of patients. And the scheduled treatment plans were changed for five out of thirteen cases despite preventive administration of antibiotics, these infections may affect the overall prognosis of the patients affected. Consequently, it will be important to clarify the route of transmission between patients, to standardize the procedure of infection measurement, and treatment strategies considering the high possibility of nosocomial infection among immunocompromised patients.
Conclusions

We report nosocomial *H. cinaedi* infection occurred in our hospital. We supposed that it is highly likely that *H. cinaedi* infections have been overlooked due to difficulties of detection in culturing the bacterium. We believe that some cases that have been diagnosed as FN may actually be septicemia caused by *H. cinaedi*. Physicians should consider the possibility of septicemia caused by *H. cinaedi*, and nosocomial transmission of *H. cinaedi*, when numerous patients receiving chemotherapy present a fever, and cellulitis.

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