Sepsis due to a novel urease-positive *Helicobacter* species in a young man

Nobuaki Mori1)#, Narito Kagawa2), Akiko Higuchi2), Yasuko Aoki3), and Kiyofumi Ohkusu3)

1) Department of General Internal Medicine, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

2) Department of Clinical Laboratory, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

3) Department of Microbiology, Tokyo Medical University, Tokyo, Japan

**Corresponding author:** Nobuaki Mori, M.D., Ph.D., Department of General Internal Medicine, National Hospital Organization Tokyo Medical Center, Tokyo 152-8902, Japan. Tel: +81-3-3411-0111; Fax: +81-3-3412-9811; E-mail: nobuaki.m@icloud.com

**Keywords:** non-*Helicobacter pylori* *Helicobacter* species; enterocolitis; bacteremia

Running title: Sepsis due to a urease-positive *Helicobacter* species
We report the first case of sepsis with enterocolitis that was caused by a novel urease-positive *Helicobacter* species in a young man. The isolates were characterized via 16S rRNA gene sequencing and their biochemical properties, and the patient was successfully treated with short-term antimicrobial therapy; no recurrence was observed.
A 21-year-old previously healthy man presented to the emergency department with abdominal pain, tenesmus and fever. His abdominal pain was initially localized to the epigastric region, and later localized in the right lower quadrant region. He did not have a medical history, and was not taking medication. He reported having no homosexual contact or pet exposure. Physical examination at the admission revealed that his blood pressure was 113/69 mmHg; his pulse rate was 92 beat per min, his temperature was 38.5°C, and his oxygen saturation was 98% in ambient air. Upon palpation, the patient had abdominal tenderness in the right lower quadrant region; no skin lesions were observed. The laboratory test results revealed leukocytosis (14,200/µL; normal range: 3,500–8,500/µL) and elevated C-reactive protein level (7.9 mg/dL; normal range: ≤ 0.4 mg/dL), although his liver and renal function was normal. Abdominal enhanced computed tomography revealed a wall thickening from the cecum to the ascending colon, with swollen mesenteric lymph nodes and a normal appendix. Two sets of blood cultures (4 bottles), in addition to urine and stool cultures were taken at the time of admission. The patient was treated with an intravenous
infusion of cefmetazole (CMZ; 1 g every 8h), based on the suspicion of bacterial enterocolitis, and his symptoms immediately improved. The patient was discharged on day 5. Although the urine and stool cultures did not yield pathogenic bacteria, the 2 aerobic bottles of blood cultures, collected on the day of admission, were found to be positive for pathogenic bacteria on day 5.

For the diagnostic testing, the Bactec FX system (Becton, Dickenson and Company [BD], Tokyo, Japan) was used for the blood culture, and positive bacterial isolates were found in only the 2 Bactec aerobic bottles after 5 days of incubation. Further testing revealed that these isolates were thin, slightly curved, Gram-negative rods. We then used the API Campy (SYSMEX BioMerieux, Tokyo, Japan) system to determine that the species was *Helicobacter pylori*. However, the colony morphology and biochemical properties of the isolates did not match those of *H. pylori* (Table 1). Therefore, samples from the bottles were subcultured in 5% sheep blood agar (Nissui, Pharmaceutical Co., Tokyo, Japan) and Skirrows medium (BD). The plates were then incubated under microaerophilic conditions at 37°C and 42°C with high humidity. After 3 days of incubation, moist, glassy, and swarming thin film colonies were
observed on both agar plates, and these colonies were also long, thin, and slightly curved to spiral-shaped Gram-negative rods. We then performed biochemical tests using the standard methods (1), and found that the isolates produced oxidase, catalase, alkaline phosphatase, and γ-glutamyltransferase, although they did not reduce indoxyl acetate or nitrate. The isolates were intermediate resistant to nalidixic acid (30 mg) and were resistant to cephalothin (30 mg). Therefore, to identify the organism, PCR amplification and sequencing was performed to analyze the 16S rRNA gene via DNA that was extracted from the isolates. Genomic DNA was extracted using the physical method with zirconia beads (Mora extraction kit; AMR Co., Gifu, Japan), according to the manufacturer’s instructions. The universal primers 8UA (5'-AGAGTTTGATCMTGGCTCAG-3') and 1485B (5'-ACGGGCGGTGTGTRC-3') were used, as previously described (2). We performed sequencing analysis (1440 base pairs) using a GenBank BLAST search and EzTaxon (http://www.ezbiocloud.net/eztaxon/). The sequence of the isolates’ 16S rRNA gene (GenBank accession no. LC028024) exhibited the greatest similarity to H. equorum (98.19%), H. canadensis (97.57%), and H. pullorum (97.44%). However, the
biochemical properties of the isolates were different from those of other Helicobacter species. Therefore, we performed antimicrobial susceptibility testing and evaluated the minimum inhibitory concentrations (MICs) for various antibiotic agents using the Dry Plate Eiken (Eiken Chemical Co., Tokyo, Japan) and the modified Levinthal agar (Nikken Bio Medical Laboratory, Kyoto, Japan). The MICs of the various antibiotic agents are listed in Table 2, and the MIC for CMZ was found to be >32 µg/mL.

The patient remained well after short-term antimicrobial therapy, without any additional treatment, until 5 months later, at which time he was admitted again for 1 week with aseptic meningitis. His blood cultures were sterile at that time.

To our best knowledge, this is the first case of sepsis with enterocolitis that was caused by a novel urease-positive Helicobacter species in a human.

Among the 33 species in the Helicobacter genus, only 10 species have been isolated from human clinical specimens: H. pylori, H. cinaedi, H. bilis, H. canadensis, H. canis, H. fennelliae, H. pullorum, H. suis, H. bizzozeronii, and H. felis (3). However, these species are associated with gastric, intestinal, and hepatobiliary conditions in humans.
In addition, a substantial number of reports have contributed to a better understanding of both human and animal infection with non-

*Helicobacter* species (NHPH) (3-5). These NHPH species are known to colonize certain animals, such as pigs and cats (6). In addition, their association with human gastroenteritis suggests that it is likely a foodborne disease and a zoonosis. Among the cases of bacteremia due to NHPH, close contact with a household pet or animals has often been reported (6, 7), although our patient reported no such contact. Alternatively, bacteremia cases caused by NHPH (such as *H. cinaedi, H. bilis, H. canis*, and *H. equorum*) have been reported in patients with underlying immunosuppressive conditions, such as acquired-immune deficiency syndrome and X-linked agammaglobulinemia (7-12). Furthermore, previous reports have described *H. cinaedi*-related bacteremia in men who have engaged in homosexual activity. Our patient denied being homosexual or engaging in homosexual activity, although it is possible that he may have lied about his sexual history. Therefore, we cannot identify the source of his infection, as we were unable to evaluate for the presence of immune disorders, as he recovered immediately. Nevertheless, we assume
that he did not have any congenital immune disorders (such as X-linked agammaglobulinemia), as he did not have any medical history.

Regarding the route of entry of Helicobacter species, we assumed the intestinal tract was the route of entry based on the clinical and imaging findings although Helicobacter species was not isolated from the stool culture. H. cinaedi has a strong ability for vascular invasion, which can result in bacterial translocation from the intestinal tract to the vascular system. Therefore, it is possible that the Helicobacter species from the present case was colonized as intestinal flora in the patient’s colon, and invaded his bloodstream through the mucosal damage that was caused by the enterocolitis. Although NHPH infections are thought to be related to specific hosts, a number of recent reports have described H. cinaedi infections in immunocompetent individuals (13, 14). Therefore, further studies are needed to determine the pathogenicity of NHPH in healthy patients.

As patients with NHPH infection have recurrent symptoms, many reports have recommended long-term antimicrobial therapy to prevent recurrent symptoms (4, 12, 15, 16). However, our patient did not experience recurrence over a 5-month period.
after short-term antimicrobial therapy, although we cannot be sure of its efficacy given
the high MIC for CMZ. Furthermore, *in vitro* susceptibility testing has seldom been
evaluated or standardized for NHPH species, as the cultures are difficult to perform and
there are no recommended guidelines for the treatment of NHPH infections. Therefore,
the selection criteria for antimicrobial agents and their treatment period must also be
established in the future.

Unfortunately, NHPH species are difficult to culture (6), and our routine
identification process originally identified the isolates as *H. pylori*, although we
subsequently discovered that the isolates’ colony formation and biochemical properties
were unique. Therefore, molecular diagnoses based on the sequencing of bacterial 16S
rRNA can be useful for rare bacteria or those that are difficult to culture. In addition,
16S rRNA is currently the first-line tool for evaluating the taxonomic status of a
prokaryotic strain at the same genus or species levels. Although the present isolates
exhibited >97% similarities to 3 *Helicobacter* species (based on their 16S rRNA gene
sequences), its biochemical properties were very unique and distinctive. According to
Stackebrandt and Ebers, two strains are members of the same species if their 16S
rRNA gene sequences are >99% identical, although a novel species may have been isolated if the sequences are <98.7% identical (17). More recently, Kim et al. have proposed a threshold of 98.65% for recognizing novel species (18). Therefore, we believe that our findings indicate that the isolate from the present case are a novel Helicobacter species. To provide other researchers with access to the isolate information, the isolate was deposited at the Japan Collection of Microorganisms, Ibaraki, Japan and given a catalogue number, JCM 30736. In the future, we expect that the isolate will be classified as a novel species based on further examination when strains with the same characteristics are isolated.

In conclusion, a previously healthy host developed bacteremia associated with enterocolitis that was caused by a novel urease-positive Helicobacter species, and a short period of antimicrobial therapy did not result in recurrence. Further studies should determine whether NHPH species are associated with diseases that are related to the host’s immunity, and identify the appropriate antibiotics and treatment periods for NHPH infections.
Acknowledgements

None.

Conflicts of interest

The authors declare no conflicts of interest are associated with this study.
Reference


Table 2. Antimicrobial susceptibilities for the patient’s isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PenicillinG</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Cefmetazole</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Flomoxef</td>
<td>32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>32</td>
</tr>
<tr>
<td>Cefepime</td>
<td>16</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>8</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>≤16</td>
</tr>
<tr>
<td>Sulbactam/cefoperazone</td>
<td>≤8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Meropeneme</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Glyndamycin</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4</td>
</tr>
<tr>
<td>Levofloxavcin</td>
<td>2</td>
</tr>
</tbody>
</table>