

## Laboratory and Epidemiology Communications

### Isolation of *Helicobacter cinaedi* from a Sepsis Patient with Cellulitis

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*Helicobacter cinaedi* is known to cause gastroenteritis, sepsis, and cellulitis in immunocompromised, and also rarely in immunocompetent, hosts (1,2). *H. cinaedi* is a fastidious

bacteria which is characterized by slow growth and the requirement of unique growth conditions. This pathogen is difficult to identify in clinical microbiology studies and hospital laboratories. In the present report, we describe the isolation of *H. cinaedi* from a sepsis patient, and we report the process of identification of this isolate using PCR product analysis.

The patient was a 58-year-old male who received mainte-

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nance hemodialysis 3 times per week due to end-stage renal disease caused by chronic glomerulonephritis. Local pain, redness, warmth, and swelling developed in the patient's right lower leg on August 25, 2006, and he developed a fever of 39°C on August 26. The patient was admitted to the hospital on August 28, 2006 and was given cefotiam at 1 g/day. Similar symptoms also developed in the patient's lower left leg on August 31. At this point, the patient was diagnosed with cellulitis. Since the fever spike (>39°C) continued, treatment was changed to the administration of 0.5 g/day of meropenem on September 1. The fever decreased transiently and the clinical findings (e.g., leukocyte count and C-reactive protein level) improved. However, a fever spike (38.3°C) was again observed on September 15. The patient was given ciprofloxacin at 200 mg/day orally and pazufloxacin mesilate at 500 mg/3-days intravenously from September 16 to September 19. The fever subsided on September 19, and the patient was discharged from the hospital on September 28.

A blood specimen was collected on August 28, and was cultured for 4 days using an automated blood culture system. A Gram-negative spiral bacterium was isolated from this culture study. The isolated bacteria was first thought to be *Helicobacter* based on its morphology, motility, and growth rate. The bacterium was examined for growth in six types of agar using a GasPak Plus anaerobic system (BBL, Sparks, Md., USA) without a catalyst, or Aneropack MicroAero (Mitsubishi GAS Chemical. Co., Tokyo, Japan). After cultivation for 4 days at 37°C, the bacteria grew well in 5% sheep's blood agar (K) (BBL), BY chocolate agar (BBL), and CDC anaerobe blood agar (BBL), but not in any of the following: TSAII 5% sheep blood agar (BBL), Anaero Columbia rabbit blood agar (BBL), or CCDA (Oxoid, Basingstoke, Hampshire, England) (Table 1). In addition, this bacterium grew better under GasPak Plus anaerobic conditions than under the conditions used with the Aneropack MicroAero system; however, it did not grow under either aerobic or anaerobic conditions at 25°C or 43°C. The bacteria were Gram-negative and rod-shaped; positive for oxidase, catalase, and reduction of nitrate; and they were negative for hippurate and indoxyl acetate. Corkscrew-shaped motility was observed.

Table 1. Growth pattern of the isolate in different media and microaerobic conditions

Media	Microaerobic conditions	
	GasPak Plus <sup>1)</sup>	Aneropack
5% sheep blood agar (K)	+++	++
BY chocolate agar	++	+
CDC anaerobe blood agar	++	+
TSAII 5% sheep blood agar	-	-
Anaero Columbia rabbit blood agar	-	-
CCDA	-	-

<sup>1)</sup> without catalyst, +++: good, ++: fair, +: slight, -: nil.

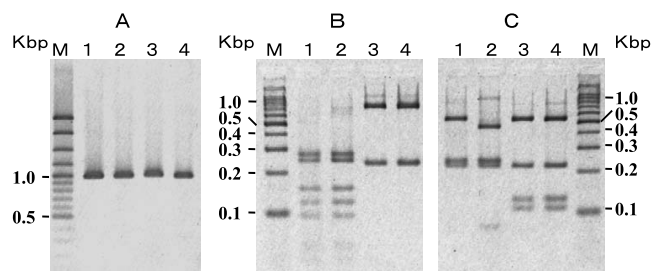


Fig. 1. PCR-RFLP analysis. (A) PCR products. (B) PCR-RFLP patterns after digestion with *DdeI*. (C) PCR-RFLP patterns after digestion with *BsrI*. Lane 1, *C. jejuni*; lane 2, *C. lari*; lane 3, ATCC BAA-847; lane 4, the isolate from the patient. Lane M, molecular marker.

Morphologically, the bacteria became round just after exposure to air. These characteristics suggest that the bacteria in the culture were *H. cinaedi*.

The bacteria were then analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP). The primer sequences were CAH 16S 1a (AAT ACA TGC AAG TCG AAC GA) and CAH 16S 1b (TTA ACC CAA CAT CTC ACG AC), according to the sequence of the 16S rRNA gene common among *Campylobacter*, *Arcobacter*, and *Helicobacter* (3). The PCR product was expected to be 1,004 bp in length, and *Campylobacter*, *Arcobacter*, and *Helicobacter* can be determined based on the restriction pattern by *DdeI*, *TaqI*, or *BsrI* digestion (Figure 1). The bacteria isolated from the patient demonstrated a restriction pattern compatible to that of the *H. cinaedi*-type strain (ATCC BAA-847). Based on these results, the isolated bacteria was confirmed to be *H. cinaedi*.

*H. cinaedi* is among those bacterial species most difficult to isolate and identify. Thus, it is possible that the importance of those bacteria in infectious diseases has been underestimated. Further study will be needed to elucidate the importance of *H. cinaedi* in infectious diseases in humans.

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## REFERENCES

1. Totten, P.A., Fennell, C.L., Tenover, F.C., et al. (1985): *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennelliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. *J. Infect. Dis.*, 151, 131-139.
2. Kitamura, T., Kawamura, Y., Ohkusu, K., et al. (2007): *Helicobacter cinaedi* cellulitis and bacteremia in immunocompetent hosts after orthopedic surgery. *J. Clin. Microbiol.*, 45, 31-38.
3. Marshall, S.M., Melito, P.L., Woodward, D.L., et al. (1999): Rapid identification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. *J. Clin. Microbiol.*, 37, 4158-4160.