

[CASE REPORT]

Vertebral Osteomyelitis Caused by *Helicobacter cinaedi* Identified Using Broad-range Polymerase Chain Reaction with Sequencing of the Biopsied Specimen

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Abstract:

A 65-year-old man presented with gradually exacerbating low back pain. Magnetic resonance imaging revealed vertebral osteomyelitis in the Th11-L2 vertebral bodies and discs. The patient showed negative findings on conventional cultures. Direct broad-range polymerase chain reaction (PCR) with sequencing of the biopsied specimen had the highest similarity to the 16S rRNA gene of *Helicobacter cinaedi*. This case suggests that direct broad-range PCR with sequencing should be considered when conventional cultures cannot identify the causative organism of vertebral osteomyelitis, and that this method may be particularly useful when the pathogen is a fastidious organism, such as *H. cinaedi*.

Key words: vertebral osteomyelitis, *Helicobacter cinaedi*, broad-range PCR with sequencing

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Introduction

Both blood and biopsy specimen cultures are useful diagnostic tests for confirming the causative pathogen of vertebral osteomyelitis. However, the positivity rates of both tests have been reported to be limited to 58% and 77%, respectively (1). Recently, broad-range 16S rRNA gene polymerase chain reaction combined with sequencing [broad-range polymerase chain reaction (PCR) with sequencing] has been used to identify the causative pathogen in cases where conventional cultures are negative (2). Using this new technique, the causative pathogen is accurately identified by gene amplification and sequencing of a broad-range gene target for bacteria (3).

We herein report the case of a 65-year-old man with vertebral osteomyelitis caused by *Helicobacter cinaedi* confirmed using broad-range PCR with sequencing that had not been identified by either blood or biopsy specimen cultures.

Case Report

The patient was a 65-year-old man without any significant medical history. He presented with gradually exacerbating low back pain for the past year. He denied a fever or any associated neurological symptoms. He did not have an immunocompromised background.

On admission, his vital signs were as follows: blood pressure, 141/73 mmHg; heart rate, 77 beats/min; body temperature, 36.3°C; respiratory rate, 20 breaths/min; and oxygen saturation on room air, 95%. A physical examination revealed knock pain at the L1 level but no abnormal neurological findings. Laboratory tests showed an elevated C-reactive protein (CRP) level (3.36 mg/dL; normal, <0.3 mg/dL) and an elevated erythrocyte sedimentation rate (41 mm/h; normal, <14 mm/h). His white blood cell count, hemoglobin level, platelet count, serum electrolyte level, plasma glucose level, liver function test results, and creatine phosphokinase level were all normal. Magnetic resonance imaging revealed findings of vertebral osteomyelitis in the Th11-L2

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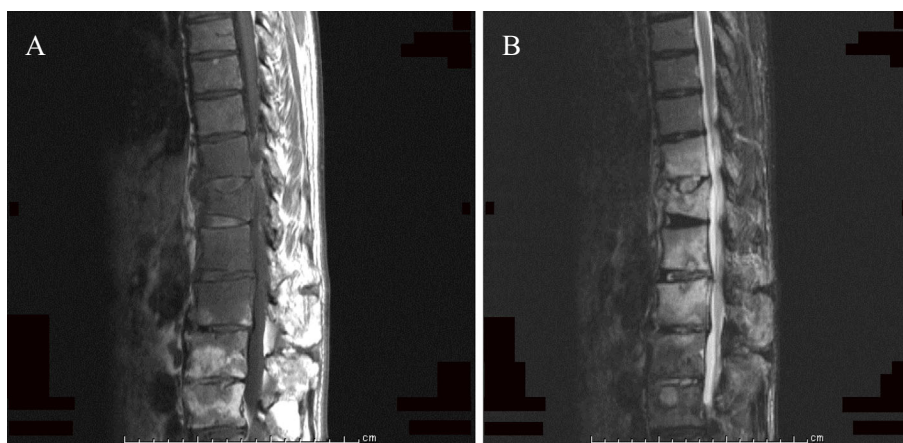


Figure. Magnetic resonance imaging. The image in A was taken with T1-weighted imaging, showing a low signal in the Th11-L2 vertebral bodies and disks. The image in B was taken with T2-weighted short-tau inversion recovery imaging, showing a high signal in the Th11-L2 vertebral bodies and disks.

Table. A Summary of Five Cases of Vertebral Osteomyelitis Caused by *Helicobacter cinaedi* Reported in the English Literature along with the Present Case.

| Case | Age, years/sex | Underlying conditions | Site of infection | Blood culture | Culture of biopsied specimen | Direct PCR of biopsied specimen | Treatment | Outcome | Reference |
|--------------|----------------|---|-------------------|---------------|------------------------------|---------------------------------|--|---------|-----------|
| 1 | 75 M | Hypertention Coronary artery disease | L5-S1 | Positive | NA | NA | Meropenem 1w Ertapenem 5w | Cure | (4) |
| 2 | 64 M | None | L5 | Positive | Negative | NA | (Cefazolin+ Fosfomycin) 8w | Cure | (5) |
| 3 | 56 M | Bronchial asthma | C6-7 | Positive | NA | NA | Ceftriaxone 6w | Cure | (6) |
| 4 | 66 M | Diabetes, Liver cirrhosis | L1-2 | Positive | Positive | Positive | Ampicillin 1w Oral Doxycycline 11w | Cure | (7) |
| 5 | 55 M | Hypertention | L4-5 | Negative | Negative | Positive | Ceftriaxone 8w | Cure | (8) |
| Present case | 65 M | Epilepsy | Th11-L2 | Negative | Negative | Positive | Ceftriaxone 6w Oral Minocycline 24w | Cure | Our case |

M: male, L: lumbar vertebra, C: cervical vertebra, Th: thoracic vertebra, NA: not applicable, w: week(s)

vertebral bodies and disks (Figure).

Two sets of blood culture were performed; however, no organism grew on culture. A percutaneous biopsy of the lumbar disk was performed. Bacteria were not observed on Gram staining. In addition, no growth was found on blood agar or chocolate II agar after 72 h of incubation at 35°C under 5% CO₂, nor in thioglycollate broth after 14 days at 35°C.

Direct broad-range PCR with sequencing of the biopsied specimen was performed. The consensus sequence had the highest similarity (1,433/1,441 bp 99.41% match in GenBank, Accession No. NR_025941, 1,431/1,438 bp 99.51% match in EzTaxon, Accession No. ABQT01000054) to the 16S rRNA gene of *H. cinaedi*.

We started the patient on ceftriaxone, and his low back pain and elevated CRP level gradually improved. We switched ceftriaxone to oral minocycline after six weeks, and he was discharged. Treatment with minocycline was

continued for approximately six months, and there have been no signs of relapse during three months of follow-up.

Discussion

The course of the present case emphasizes two important clinical points. First, *H. cinaedi* should be suspected as a pathogen in vertebral osteomyelitis, even when conventional cultures are negative. Second, broad-range PCR with sequencing of biopsy specimens is particularly useful when conventional cultures cannot identify the causative organism of vertebral osteomyelitis.

H. cinaedi should be suspected as a pathogen in vertebral osteomyelitis even when conventional cultures are negative. Six cases of vertebral osteomyelitis with *H. cinaedi* have been reported, and two of these cultures were negative, including our case (Table) (4-8). *H. cinaedi* was detected on blood culture in four cases. A biopsy was performed in four

cases, and the biopsy specimen culture was positive in only one case. In two cases, including our present case, broad-range PCR with sequencing directly performed using the biopsy specimen confirmed the presence of *H. cinaedi*, although the culture was negative. Several reasons for the inability to detect the causative organism have been suggested. First, prior antimicrobial exposure decreases the sensitivity of the conventional culture. However, in our case, no antibiotic had been used prior to performing culture. Second, fastidious organisms, including *H. cinaedi*, are difficult to culture. The detection of *H. cinaedi* is known to be particularly difficult because it rarely grows on traditional culture media (9). Nonselective media incubated in a microaerobic condition (5%O₂), ideally with hydrogen gas (5-10%), or selective media such as Skirrow or Butzler Blaser were reported to be suitable for the isolation of *H. cinaedi* (9, 10). In our case, we used neither a microaerobic condition nor selective media because we did not suspect *H. cinaedi* to be the causative organism. In addition, *H. cinaedi* grows slowly under microaerobic conditions; therefore, a long incubation period is recommended for its detection. Four to ten days are considered necessary for a positive result with a culture bottle of an automatic blood culture system (10). Given the findings of this previous report, we prolonged the incubation time of blood culture to 10 days; however, we did not observe any organism growth.

The optimum antibiotic selection and duration for vertebral osteomyelitis with *H. cinaedi* have yet to be determined. Indeed, the selection of antimicrobials has varied in previous case reports (Table). We selected ceftriaxone and oral minocycline based on a previous report of successful treatment with these drugs for *H. cinaedi* bacteremia (11). A treatment duration of more than six weeks in all cases achieved favorable outcomes.

The present case suggests that broad-range PCR with sequencing of biopsy specimens is particularly useful when the causative organism of vertebral osteomyelitis cannot be identified using conventional cultures. Choi et al. reported that broad-range PCR with sequencing is more sensitive than routine culture for the etiological diagnosis of vertebral osteomyelitis (12). In their study, broad-range PCR with sequencing confirmed the causative organism in 16 cases of vertebral osteomyelitis in which biopsy specimen culture had been negative. Given that three-quarters of the patients had been treated with antimicrobial agents, the authors concluded that broad-range PCR would be particularly useful for patients with a history of antimicrobial therapy. The findings of our case further imply that this method might be useful when the pathogen is a fastidious organism, such as

H. cinaedi.

In conclusion, we encountered a rare case of vertebral osteomyelitis caused by *H. cinaedi* identified using broad-range PCR with sequencing of a biopsy specimen that had not been detected with conventional culture. The findings of our case suggest that performing broad-range PCR with sequencing should be considered for the detection of the causative organism when blood or biopsy specimen culture fails.

The authors state that they have no Conflict of Interest (COI).

References

- Zimmerli W. Clinical practice. Vertebral osteomyelitis. *N Engl J Med* **362**: 1022-1029, 2010.
- Rampini SK, Bloemberg GV, Keller PM, et al. Broad-range 16S rRNA gene polymerase chain reaction for diagnosis of culture-negative bacterial infections. *Clin Infect Dis* **53**: 1245-1251, 2011.
- Petti CA. Detection and identification of microorganisms by gene amplification and sequencing. *Clin Infect Dis* **44**: 1108-1114, 2007.
- Smith G, Amber N, Wolk DM, Hoover SE. *Helicobacter* vertebral osteomyelitis in an immunocompetent adult. *Infect Dis Clin Pract* **20**: 61-62, 2013.
- Haruki Y, Hagiya H, Hashimoto T, et al. Pyogenic spondylitis and diskitis caused by *Helicobacter cinaedi* in an immunocompetent adult patient. *Intern Med* **54**: 2415-2418, 2015.
- Murata S, Suzuki H, Sakamoto S, et al. *Helicobacter cinaedi*-associated vertebral osteomyelitis in an immunocompetent patient. *Intern Med* **54**: 3221-3224, 2015.
- Yoshizaki A, Takegawa H, Doi A, Mizuno Y, Nishioka H. Vertebral osteomyelitis caused by *Helicobacter cinaedi*. *J Clin Microbiol* **53**: 3054-3056, 2015.
- Sekikawa Y, Hongo I, Oda R, Ohkusu K. *Helicobacter cinaedi* vertebral osteomyelitis identified by 16S rDNA analysis of tissue in an immunocompetent adult [abstract]. *Open Forum Infect Dis* **2**: 1516, 2015.
- Matsumoto T, Goto M, Murakami H, et al. Multicenter study to evaluate bloodstream infection by *Helicobacter cinaedi* in Japan. *J Clin Microbiol* **45**: 2853-2857, 2007.
- Kawamura Y, Tomida J, Morita Y, Fujii S, Okamoto T, Akaike T. Clinical and bacteriological characteristics of *Helicobacter cinaedi* infection. *J Infect Chemother* **20**: 517-526, 2014.
- Uwamino Y, Muranaka K, Hase R, Otsuka Y, Hosokawa N. Clinical features of community-acquired *Helicobacter cinaedi* bacteremia. *Helicobacter* **21**: 24-28, 2016.
- Choi SH, Sung H, Kim SH, et al. Usefulness of a direct 16S rRNA gene PCR assay of percutaneous biopsies or aspirates for etiological diagnosis of vertebral osteomyelitis. *Diagn Microbiol Infect Dis* **78**: 75-78, 2014.

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