Escherichia coli Vertebral Osteomyelitis Diagnosed According to Broad-range 16S rRNA Gene Polymerase Chain Reaction (PCR)

Satoshi Shibata¹, Ryutaro Tanizaki¹, Koji Watanabe¹, Kenta Makabe², Naoki Shoda², Satoshi Kutsuna³, Maki Nagamatsu⁴, Shinichi Oka¹ and Norio Ohmagari¹

Abstract

Identifying the causative agent of pyogenic osteomyelitis is often challenging, especially when antibiotics are administered before a biopsy. We herein present a case of osteomyelitis in the cervical vertebrae presenting with progressive paralytic symptoms, in which we successfully identified Escherichia coli from a biopsy specimen using broad-range 16S rRNA gene polymerase chain reaction (PCR) even though sensitive antibiotics had been used for more than 50 days before the biopsy. Broad-range 16S rRNA gene PCR is a useful diagnostic method, especially when prebiopsy antibiotics are unavoidably used for a clinically unstable state.

Key words: a broad-range 16S rRNA gene PCR, Escherichia coli (E. coli), vertebral osteomyelitis, prebiopsy antibiotics exposure

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Introduction

Identifying the causative agent of pyogenic osteomyelitis is often challenging, especially when antibiotics are administered before performing a biopsy of the involved lesion. We experienced the case of a patient presenting with complete paraplegia resulting from osteomyelitis of the cervical vertebrae in which Escherichia coli (E. coli) was identified as a causative pathogen based on broad-range 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) followed by detected sequencing (broad-range 16S rRNA gene PCR).

Case Report

A 58-year-old woman was referred to our hospital with a 1-month history of cervical back pain, fever, progressive symmetric weakness of both limbs and bladder and rectal disturbance (BRD) (day 0). More than two months before referral (day -82), she developed bacteremia caused by E. coli resulting from a urinary tract infection and was treated with cefmetazole (CMZ) for 17 days at a local hospital. The E. coli, which was isolated from blood and urine cultures, was susceptible to ampicillin/sulbactam, cefazolin, CMZ, ceftazidime, meropenem and amikacin and resistant to piperacillin and levofloxacin. Thereafter, the fever and pyuria reappeared, and bilateral renal abscesses were identified on magnetic resonance imaging (MRI) (day -52). Antibiotic treatment (intravenous or oral cephalosporin) was administered intermittently depending on the patient’s symptoms, such as fever and pyuria (Fig. 1). During this period, she developed progressive cervical back pain, followed by symmetric weakness of both limbs and BRD. MRI of the cervical spine was performed on day -33, which showed osteomyelitis accompanied with discitis of C5 and C6 surrounded by an epidural abscess compressing the spinal cord (Fig. 2). She was transferred to our hospital for surgical procedures (day 0). On admission, she showed complete paraplegia of both legs without a fever. She had no past history other than

¹AIDS Clinical Center, National Center for Global Health and Medicine, Japan, ²Department of Orthopedic Surgery, National Center for Global Health and Medicine, Japan, ³Disease Control and Prevention Center, National Center for Global Health and Medicine, Japan and ⁴Department of Infectious Disease, Research Institute, National Center for Global Health and Medicine, Japan

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Correspondence to Dr. Satoshi Shibata, flush-ss@hotmail.co.jp
diabetes mellitus type 2 without medication. Treatment with intravenous cefepime (CFPM: 2 g every 8 h) and vancomycin (VCM: 1 g every 12 h) was started as an empiric therapy. Blood cultures performed at referral were negative. Saucerization of infected bone with bone grafting on day 11, laminectomy of C5-C6 and drainage of the spinal epidural abscess were performed on day 18. No visible organisms were identified on a Gram-stained smear of the abscess material, although it showed a considerable number of polymorphonuclear leukocytes. Cultures of the abscess and soft tissue were also negative. Therefore, we performed molecular identification with PCR amplification and a sequence analysis of the 16S rRNA gene. Bacterial DNA was extracted from homogenized biopsy samples (abscess and soft tissue) using the DNA extraction kit according to the manufacturer’s instructions (MORA-EXTRACT, Kyokuto Pharmaceutical Industrial, Tokyo, Japan). The 16S rRNA gene was amplified using the PCR primers; 5’-TTGGAGAGTTTGATCTTGCTC-3’ and 5’-ACGGCGGTGTTGRTC-3’ (1, 2). Thereafter, the purified PCR products were sequenced with Big Dye terminator reagents on an ABI 3130XL genetic analyzer (Applied Biosystems, Carlsbad, USA). We performed a homologous comparison using a GenBank BLAST search and the EzTaxon server (http://www.ezbiocloud.net/eztaxon) phylogenetic tools. Sequences of the 16S rRNA gene amplified from the abscess and soft tissue showed 99.92% identity (1,267 bp over the entire 1,269-bp fragment) and 100% identity (1,144 bp over the entire 1,144-bp fragment) to that of *E. coli* (KCTC 2441T; accession number EU014689). Based on these results, we determined the isolates to be *E. coli*. Nucleotide sequences of the abscess and soft tissue have been deposited in DDBJ under accession numbers LC037388 and LC037389.

Referring to the susceptibility of the *E. coli* cultured from urine and blood obtained at the former hospital, CFPM and VCM were switched to ceftriaxone (CTRX) (2 g q12 h) on day 24. The patient completed a 6-week course of intravenous antibiotic treatment, although she developed *Clostridium difficile* colitis and was treated with oral VCM during the therapy. She remained clinically stable, and abscess volume reduction was confirmed on MRI on day 68, although the paraplegia in both legs and BRD remained.

**Discussion**

Identifying the causative pathogen of pyogenic vertebral osteomyelitis is important for the optimal use of antibiotics. However, blood cultures often show negative results due to their low sensitivity (range across studies, 30 to 78%) (3). Cultures of biopsy specimens, obtained via CT-guided or surgical techniques, have great diagnostic value, as their sensitivity is generally higher than that of blood cultures (ranging from 47 to 100%) (3). Prebiopsy antibiotic exposure may enhance the false negative rate of cultures. Several studies have shown that the diagnostic yield of cultures of
biopsy specimens is diminished by antecedent antibiotic use (4-6). The discontinuation of antibiotics at least 48 hours prior to biopsies is generally recommended if the patient’s condition is clinically stable (7). *E. coli* is the second most frequent causative agent of vertebral osteomyelitis, which is typically present after urinary tract infections (7). Hence, in most cases of osteomyelitis caused by this pathogen, antibiotics have already been administered when symptoms of vertebral osteomyelitis emerge (8). The present patient showed progressive paralytic symptoms at referral, despite the use of cefotaxime (CTX). In view of the fact that *E. coli* was cultured from both urine and blood at the former hospital, *E. coli* was the most highly suspected pathogen. However, 40 days had already passed at the time at which she developed cervical back pain since the onset of *E. coli* bacteremia. Other pathogens causing catheter-related infections should have been considered as causative agents because she was being treated with a peripheral intravenous line at that time. For these reasons, we were unable to discontinue the antibiotics before the biopsy, and identifying the causative agent is absolutely imperative for the optimal use of antibiotics. We continued antibiotics with VCM in addition to CFPM as an empiric therapy at referral, which resulted in negative results on the Gram-stained smear and culture of the biopsy specimen. We subsequently performed broad-range 16S rRNA gene PCR using a biopsy specimen to identify the causative agent. Several previous studies have demonstrated the usefulness of broad-range 16S rRNA gene PCR for obtaining the diagnosis of normally aseptic organ infections, such as endocarditis, arthritis and vertebral osteomyelitis (9-15), although culture methods remain a reliable standard method for diagnosing infectious diseases (7). Moreover, the high priority of broad-range 16S rRNA gene PCR is indicated by the false-negative results in culture due to preceding antibiotic treatment before the biopsy (16). Table shows a clinical summary of cases of osteomyelitis diagnosed with broad-range 16S rRNA gene PCR. PCR generally has higher sensitivity than cultures, even in cases involving exposure to antibiotics prior to biopsy (17-25). In the current case, surprisingly, broad-range 16S rRNA gene PCR amplified *E. coli*-specific DNA from the biopsy specimen despite the preceding administration of susceptible antibiotics for more than approximately 50 days. Moreover, the negative results for other pathogens on “broad-range” 16S rRNA gene PCR made it possible to differentiate other potential pathogens. These results emphasize the utility of broad-range 16S rRNA gene PCR in difficult clinical settings.

On the other hand, this method has some limitations, such as difficulty in differentiating the causative agent of infection from colonization with environmental bacteria. Moreover, the interpretation of broad-range 16S rRNA is dependent on the results of a similarity search of the sequence database, which cannot be accurately identified with phenotypic tests in microbiology laboratories. More detailed guidelines for the interpretation of sequence data are expected for the more widespread use of this method in the clinical setting.

In conclusion, broad-range 16S rRNA gene PCR is a useful diagnostic method, especially when prebiopsy antibiotics are unavoidably used for a clinically unstable state. Further clinical evidence of the sensitivity and specificity of broad-range 16S rRNA gene PCR in the clinical setting is warranted.

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References