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

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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

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Table. Clinical Summary of Cases with Osteomyelitis Microbiologically Diagnosed with 16S rRNA.

Reference	Clinical diagnosis	Number of cases (prior antibiotic treatment)	Duration of prior antibiotics	PCR positive / Culture positive	Organism
17	vertebral osteomyelitis	45 (29)	11.0 days [1-115 days]	24 cases (53.3%) / 13 cases (28.9%)	<i>S. aureus</i> 9 cases, <i>S. epidermidis</i> 3 cases, <i>E. coli</i> 2 cases, <i>S. agalactiae</i> 2 cases, others 8 cases, negative 21 cases
18	bone or joint infection	525 (na)	-	112 cases (21.3%) / 105 cases (20%)	<i>Staphylococci</i> 53 cases, <i>Streptococci</i> 11cases, <i>enterobacteriae</i> 11cases, HACEK 3 cases, others 3 cases (only congruent results between culture and PCR)
19	spinal infection	18	2 days	11 cases (61.1%) / 9 cases (50.0%)	<i>S. aureus</i> 7 cases, <i>S. epidermidis</i> 1 case, <i>Streptococcus</i> 2 cases,
20	spondylodiscitis	19 (0)	-	19 (100%) / 14 (73.7%)	<i>Staphylococci</i> 12 cases, <i>Streptococci</i> 2 cases, <i>Actinomyces israelii</i> 2 cases, others 3 cases
21	arthritis with osteomyelitis	1 (1)	14 days	positive / negative	<i>Streptococcus Pyogenes</i>
22	osteomyelitis of the ulna	1 (0)	-	positive / negative	<i>Prophyromonas gingivalis</i>
23	osteomyelitis	1 (0)	(antibiotic treatment was discontinued 6 months before biopsy)	positive / negative	<i>Helicobacter rappini</i>
24	osteomyelitis	1 (0)	-	positive / negative	<i>Mycoplasma pneumoniae</i>
25	osteomyelitis of the patella	1 (1)	-	positive / negative in normal procedure, BCYE was positive	<i>Legionella anisa</i>

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 wide 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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