Open Journal of
Clinical & Medical
Case Reports

Volume 2 (2016)
Issue 21

ISSN 2379-1039

Vertebral *Aggregatibacter Aphrophilus* Osteomyelitis Identified Using 16S Ribosomal RNA Sequencing Method

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Abstract

Aggregatibacter aphrophilus is a fastidious gram-negative coccobacillus, known to be difficult to identify. Recent studies have shown that extended blood culture incubation is unnecessary. We present a case of thoracic vertebral osteomyelitis and spinal epidural abscess caused by *A. aphrophilus* in a 62-year old who presented with acute fever, back pain, and urinary incontinence. MRI confirmed osteomyelitis and epidural abscess. Emergent laminectomy was performed due to nerve compression and the abscess was cultured. The blood culture became positive on day seven of cultivation, while the abscess culture became positive on day three. *A. aphrophilus* was identified by 16S ribosomal RNA sequencing method. The routine five-day blood culture incubation period might not be sufficient for *A. aphrophilus* to grow. The 16S rRNA sequencing method can be helpful when conventional phenotypic method cannot identify fastidious organisms.

Keywords

Aggregatibacter aphrophilus; thoracic vertebral osteomyelitis; 16S ribosomal RNA sequencing method

Introduction

Aggregatibacter genus is a member of the HACEK group of organisms, which also includes *Haemophilus*, *Cardiobacterium*, *Eikenella*, and *Kingella* species. *Aggregatibacter aphrophilus* was first described by Khairat in 1940 in association with a fatal case of endocarditis [1]. Although it is well known that *Aggregatibacter* species may take longer to grow in cultures, Petti et al. and Baron et al. concluded that extended incubation of blood cultures to recover HACEK was not necessary [2]. Therefore, the HACEK group of organisms is now considered to grow 5 days into the standard blood culture. Here we here present a case of thoracic vertebral osteomyelitis caused by *Aggregatibacter aphrophilus*, in which the blood culture became positive on day 7. Identification of this organism has proven to be difficult using conventional phenotypic method; however, the 16S ribosomal RNA (rRNA) sequencing method can be a useful tool for identification.

Case Report

A 62-year-old man with impaired glucose tolerance presented with fever and back pain. His back pain started 5 days before admission, and a fever of 100.4°F developed 3 days before admission. On the day of admission, the back pain caused moderate distress. Physical examination revealed a blood

pressure of 132/80 mmHg, pulse rate of 94 beats/min, respiratory rate of 12 breaths/min, temperature of 100°F, and arterial oxygen saturation of 99% on ambient air. He also had left thoracic paraspinal tenderness and decreased muscle strength of the left leg. Laboratory tests revealed a leukocyte count of 12,800 cells/μL, C-reactive protein level of 28.9 mg/dL and erythrocyte sedimentation rate of 129mm/h. Urinary retention was confirmed using a Foley catheter. Magnetic resonance imaging demonstrated decreased signal intensity at the level of T8/T9 onT1-weighted (Figure1) and increased signal intensity of the epidural cavity around T8/T9 on T2-weighted images (Figure2). Thus, vertebral osteomyelitis and epidural abscess was confirmed. Two sets of blood culture were obtained, and emergent laminectomy was performed because of nerve compression. Furthermore, cefazolin was started empirically after a culture of the abscess was obtained during surgery. The abscess culture became positive on day 3, and Gram staining revealed Gram-negative rods (GNR; Figure3). The antibiotics were changed to ampicillin/sulbactam. *A.aphrophilus* was identified from the GNR using the 16S rRNA sequencing method, and ampicillin/sulbactam was continued according to susceptibility analysis. The two sets of blood cultures obtained on admission became positive on day 7 with the same organism. Transthoracic echocardiogram revealed no evidence of vegetation, and the findings did not meet the modified Duke's criteria. After surgery and antibiotic treatment, the back pain and fever gradually improved. The antibiotics was switched to oral ciprofloxacin after a total of 6 weeks of intravenous antibiotics, which were continued until the C-reactive protein and erythrocyte sedimentation rate normalized. The patient was successfully treated with a total of 22 weeks of antibiotic therapy. His oral hygiene was good and the point of entry of the organism was not known. There has been no recurrence or residual neurological symptoms after 7 months of follow-up.

Discussion

A.aphrophilus, a fastidious Gram-negative coccobacillus, now includes species formerly known as *Haemophilus aprophilus* and *Haemophilus paraphrophilus*. Both *H. aprophilus* and *H. paraphrophilus* belong to the so-called carbon dioxide-requiring species of the *Haemophilus* genus. *H.aphrophilus* also requires X factor, but not V-factor, for growth. Selective media are required for the detection of these small, slow-growing colonies, which otherwise would be overgrown by other organisms [3].

A. aphrophilus is a part of the normal oropharyngeal flora and can be found in approximately 35% of the normal population [4]. Although generally non-pathogenic, it has occasionally been reported to cause endocarditis, sinusitis, pneumonia, brain abscess, and vertebral osteomyelitis, and dental procedures are a known cause for bacterial entry into the bloodstream [5]. In particular, endocarditis, osteoarticular infection, and brain abscess associated with *A. aphrophilus* were reported to be relatively common [6,7]; however, thoracic vertebral osteomyelitis has been rarely reported.

Recent studies have found that HACEK organisms can be easily isolated using current blood culture systems when incubated for at least 5 days, although the HACEK group is traditionally known to take longer to grow in culture. Baron et al. concluded that extended incubation periods were not necessary for the recovery of fastidious agents that cause septicemia, based on data from 215 patients suspected of having endocarditis, in which all 24 HACEK organisms grew within the standard 5-day blood culture incubation periods [8]. Petti et al. reported that of 407 blood cultures of patients suspected of culture-negative endocarditis, none grew HACEK or any other bacteria using extended incubation [2].

The mean and median periods for the detection of HACEK isolates from blood cultures have been reported to be 3.4 and 3 days, respectively. In our patient, however, the blood cultures became positive on day 7, whereas abscess culture became positive on day 3. Another recent case report has also demonstrated positive cultures after 5 days [9]. Our experience with this case suggested that standard culture monitoring limited to 5 days is insufficient for *A. aphrophilus* growth. Therefore, the incubation period of blood cultures, especially for fastidious organisms, may need to be re-considered.

The 16S rRNA sequencing method has been reported to be effective in identifying fastidious GNR such as *A. aphrophilus*, which cannot be readily identified using conventional phenotypic methods. de Melo Oliveira et al. reported that the 16S rRNA sequencing method identified 148 of 158 (94%) isolates at the species level, whereas phenotypic identification correctly identified 64 of the 158 (40%) isolates at the species level [10]. Riggio et al. concluded that the 16S rRNA sequencing method was useful for distinguishing between *H. aphrohilus* and *H. paraphrophilus* [11]. Similarly, *A. aphrophilus* was identified using the 16s rRNA sequencing method in the present case. Matrix-assisted laser desorption ionization-time off light mass spectrometry (MALDI-TOF MS) would also be a useful tool to identify HACEK isolates clinically [12]. Although MALDI-TOF MS is considered potentially time- and cost-intensive, previous studies examining the HACEK group are limited, and further investigation is needed.

Conclusion

we encountered a case of thoracic vertebral body osteomyelitis and spinal epidural abscess caused by *A. aphrophilus*, in which the blood culture became positive on day 7 of cultivation, and where the organism was identified using the 16S rRNA sequencing method. The standard 5-day cultivation of blood culture may not be sufficient for identification of *A. aphrophilus*. The 16S rRNA sequencing method is thus effective in identifying fastidious GNRs.

Figures

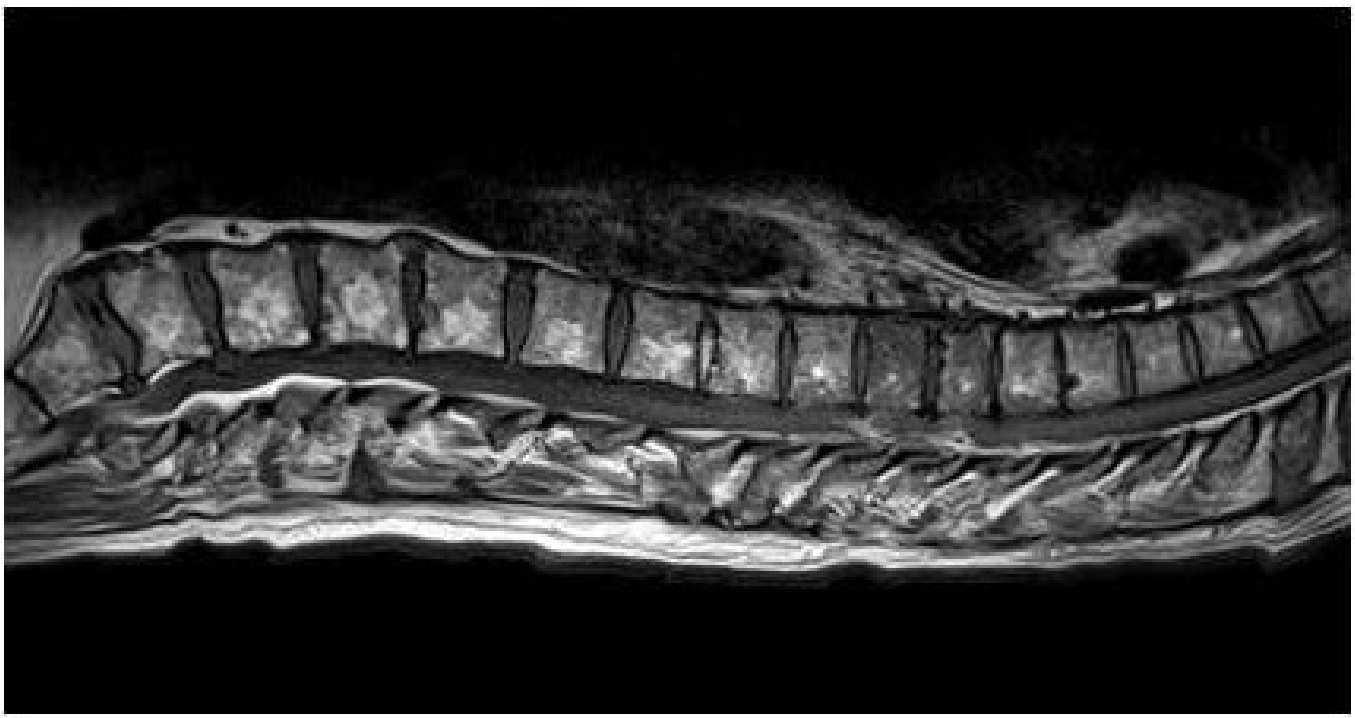

Figure 1: Magnetic resonance imaging without contrast (sagittal view).
Decreased signal intensity at the level of T8/9 in T1-weighted images.

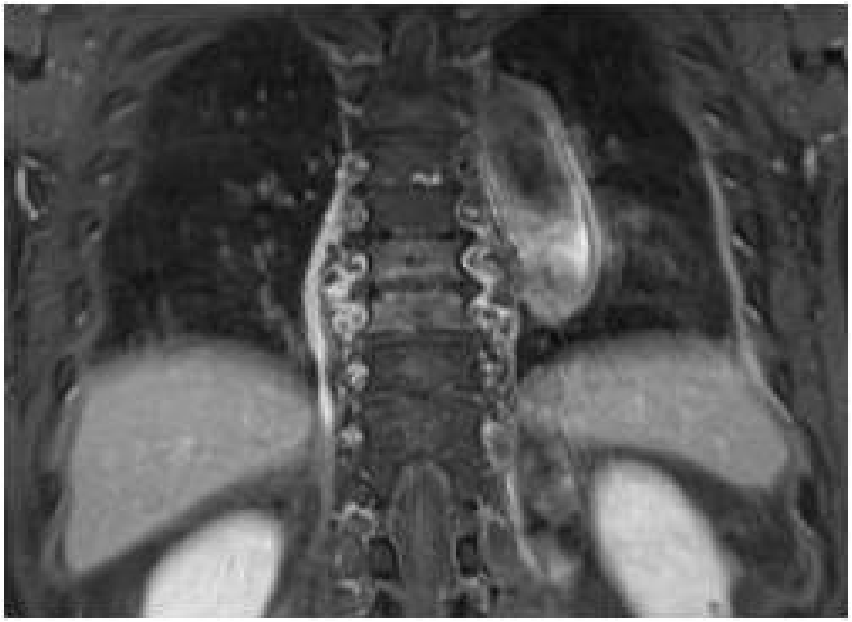

Figure 2: Magnetic resonance imaging without contrast (coronal view).
Increased signal intensity of the epidural cavity at level T8/9 in T2-weighted images.

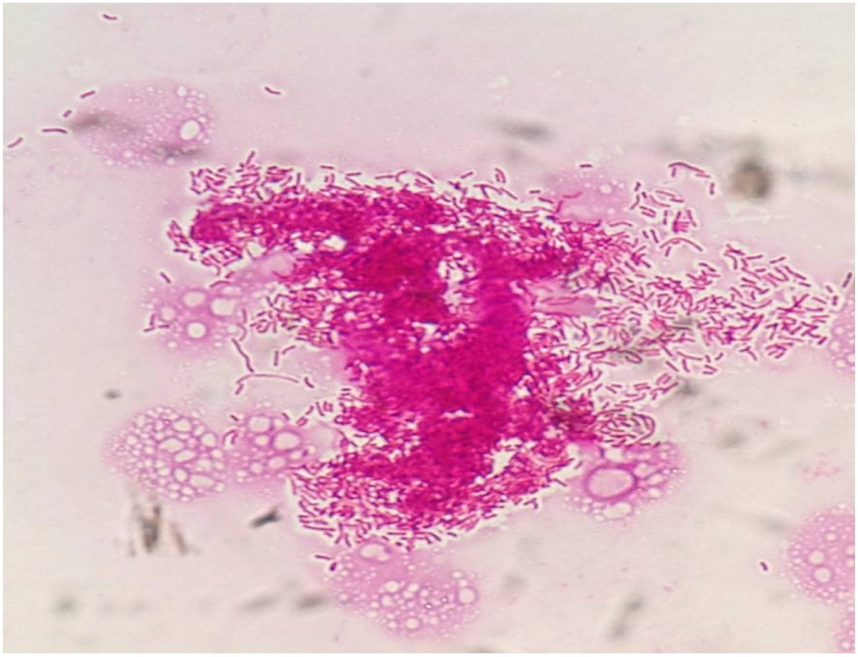

Figure 3: Gram stain of positive abscess culture (×1000)
Gram stain of the positive abscess culture showing Gram-negative rods.

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Manuscript Information: Received: July 19, 2016; Accepted: November 01, 2016; Published: November 03, 2016

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Citation: Kobayashi T, Moddy S, Hase R, Komori M, Ohkusu K, Otsuka Y, et al. Vertebral *aggregatibacter aphrophilus* osteomyelitis identified using 16S ribosomal RNA sequencing method . Open J Clin Med Case Rep. 2016; 1181

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