

## CASE REPORT

# Culture-Negative *Fusobacterium nucleatum* Brain Abscess and Pleural Empyema Cases Revealed by 16S rRNA Sequencing

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### SUMMARY

**Background:** We reported two *Fusobacterium nucleatum* cases each of brain abscesses and pleural empyema, using 16S rRNA sequencing technology.

**Methods:** We reviewed clinical records and microbiological findings in four patients with *F. nucleatum* infection.

**Results:** All conventional culture results from peripheral blood, cerebrospinal fluid, and pleural fluid samples were found to be negative for this pathogen. Three patients were treated with antimicrobial agents for more than a week before specimen sampling. All patients recovered from their fusobacterial infections and were discharged.

**Conclusions:** Molecular identification methods such as 16S rRNA sequencing should accompany conventional culture to detect obligate anaerobic bacteria in deep-seated sites and organs.

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#### KEYWORDS

*Fusobacterium nucleatum*, brain abscess, lung empyema, 16S rRNA

#### INTRODUCTION

Although culture remains the most commonly used method for identifying pathogens, some fastidious organisms causing various infections are sometimes not detected by the culture methods, especially when previously treated with antimicrobials [1,2]. *Fusobacterium nucleatum* is an oral commensal as well as a pathogen associated with pregnancy related complications and gastrointestinal disorders in addition to other infections [3]. Owing to its anaerobic characteristic, the culture-independent method is recommended for detecting this pathogen [3]. 16S rRNA sequencing revealed an increase of 5% in the number of *Fusobacterium* infection cases in addition to those detected by the conventional culture methods [4]. The sequencing approach has enhanced sensitivity for cases previously treated with antimicrobials [5]. In Korea, *F. Nucleatum* causing liver abscess and osteomyelitis has been reported [6,7], and in two cases culture-negative *F. nucleatum* infection has

been identified in cerebrospinal fluid [8]. In the present study, we reported two culture-negative *F. nucleatum* cases, each of brain abscesses and pleural empyema, detected using 16S rRNA sequencing. The institutional review board of Asan Medical Center waived the need for review (#2022-0542).

### CASE PRESENTATION

These four fusobacterial cases are summarized in Table 1. The two patients with brain abscesses were a 71-year-old male (patient 1) and a 76-year-old female (patient 2). The two patients with pleural empyema were 53- and 15-year-old males (patients 3 and 4, respectively). Both patients with brain abscess and one with pleural empyema (patient 4) had severe periodontitis, which was the potential source of invasive fusobacterial infection. The other patient with pleural empyema (patient 3) had a previous history of bronchiectasis. Radiological examinations of the brain with magnetic resonance imaging (Figure 1A and 1B) and chest with computed tomography (Figure 1C and 1D) revealed the brain abscess and pleural effusions, respectively. All conventional culture results from peripheral blood, cerebrospinal fluid, and pleural fluid of four specimens were found to be negative for *F. nucleatum*. However, *F. nucleatum* was identified at species level using PCR amplification and 16S rRNA gene sequencing [2,9] from the same specimens of cerebrospinal or pleural fluid of all the four patients. Three patients (patients 1, 2, and 4) were treated with antimicrobial agents for more than a week before specimen sampling. Antimicrobial treatment prior to admission of patient 3 was not confirmed. Both patients with brain abscess were treated with metronidazole and ceftriaxone. The treatment was continued even after *F. nucleatum* identification. Stereotactic abscess aspiration was performed for both patients. Patient 3 was treated with cefepime, ciprofloxacin, and piperacillin/tazobactam during hospitalization and was discharged with cefditoren pivoxil and ciprofloxacin prescriptions. After identifying *F. nucleatum*, the antimicrobial treatment was changed to amoxicillin/clavulanate and ciprofloxacin. Patient 4 underwent video-assisted thoracic surgery to drain the pleural effusion in the whole thorax and was initially treated with piperacillin/tazobactam and vancomycin for a week and with cefepime and vancomycin during the following week. After *F. nucleatum* identification, the antimicrobial treatment was changed to piperacillin/tazobactam for two weeks and then amoxicillin/clavulanate for one week until discharge. All patients recovered from their fusobacterial infections and were discharged.

### DISCUSSION

Invasive fusobacterial infections such as bacteremia, peritonitis, pleurisy, and head and neck infection by *F. nucleatum* have been reported in previous studies [10-12]. All four cases in the current report were invasive fusobacterial infections detected in cerebrospinal fluid or pleural fluid. Treatment of these infections, such as meningitis and brain abscess in cases of *F. nucleatum* in several studies, was mainly accompanied by surgical treatment, with favorable outcomes [11]. In addition, two cases reporting empyema and lung abscess also showed favorable outcomes [12]. In this report, culture-negative pleural empyema and brain abscess cases caused by *F. nucleatum* responded well to antibiotic treatment and surgical intervention, if required.

In the present study, *F. nucleatum* causing brain abscess and pleural empyema were not identified using conventional culture methods, but were revealed by 16S rRNA gene PCR sequencing. Furthermore, three of the four cases in this report were treated with antimicrobials prior to specimen sampling. Therefore, negative results upon conventional culture could probably be affected by the obligate anaerobic characteristic of *F. nucleatum* and prior antimicrobial treatment history [5]. *F. nucleatum* was previously susceptible to penicillin, piperacillin/tazobactam, cephalosporin, imipenem, clindamycin, moxifloxacin, chloramphenicol, and metronidazole in the study conducted in Korea [10]. Definite species identification using 16S rRNA sequencing is also helpful in antimicrobial therapy, as shown in de-escalation of antimicrobials in the two patients with pleural empyema. Considering drawbacks of broad-spectrum antimicrobials, such as drug-associated side effects, increased costs, and the risk of bacterial resistance emergence [13], targeted antimicrobial therapy using 16S rRNA sequencing may be a highly acceptable and reliable method.

Despite increased sensitivity, the false-positive risk of 16S rRNA sequencing remains an obstacle. Contamination risk throughout all procedures, including extraction and sequencing, can cause false-positive results [14]. Therefore, all 16S rRNA sequencing result should be carefully interpreted with rigorous review of clinical features of the patients. For all patients under observation the clinical features were well correlated with the 16S rRNA sequencing results.

In conclusion, molecular identification methods, such as 16S rRNA sequencing and recently introduced targeted next-generation sequencing of 16S-23S rRNA, should accompany conventional culture to detect obligate anaerobic bacteria in the deep-seated sites and organs.

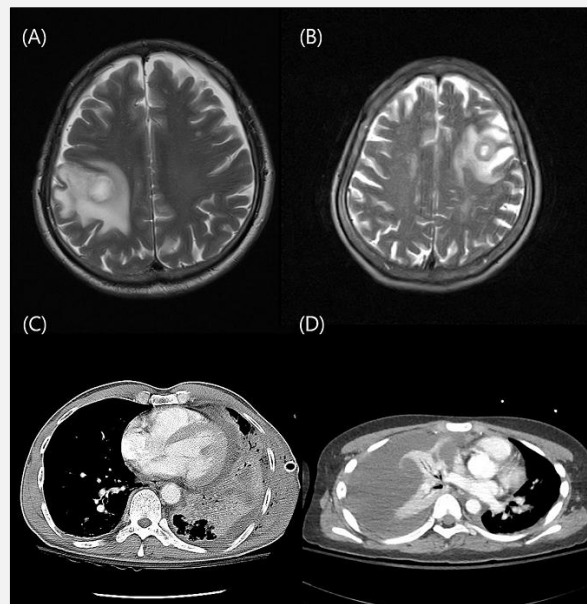
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**Table 1. Clinical features of four cases of culture-negative *Fusobacterium nucleatum* infection.**

Case no.	Age/gender	Clinical manifestation	Past medical history	Imaging findings	Surgical treatment	Antimicrobial treatment	Outcome
1	71/M	left upper extremity weakness	periodontitis	brain MRI: brain abscess, right parietal lobe	stereotactic aspiration of brain abscess	IV: ceftriaxone, metronidazole	recovered
2	76/F	left facial palsy, motor aphasia	periodontitis	brain MRI: brain abscess, left frontal lobe	stereotactic aspiration of brain abscess	IV: ceftriaxone, metronidazole	recovered
3	53/M	chest pain, fever	bronchiectasis	chest CT: multiloculated pleural effusion, left necrotizing pneumonia, left lower lobe	not applicable	IV: cefepime, ciprofloxacin, piperacillin/tazobactam PO: cefditoren pivoxil, amoxicillin/clavulanate, ciprofloxacin	recovered
4	15/M	fever after pamidronate treatment due to osteoporosis	ALL, L1 Lennox-Gastaut syndrome Phenylketonuria, BH4 deficiency periodontitis	chest CT: pleural effusion with atelectasis, right	video-assisted thoracic surgery for pleural effusion	IV: cefepime, piperacillin/tazobactam, vancomycin PO: amoxicillin/clavulanate	recovered

Abbreviations: ALL - acute lymphoblastic leukemia, BH4 - tetrahydrobiopterin, MRI - magnetic resonance imaging, CT - computed tomography, IV - intravenous, PO - per os.



**Figure 1. Radiologic findings of four patients with *F. nucleatum* infections.**

(A) Brain abscess in the right parietal lobe, observed via brain magnetic resonance imaging (MRI; T2 weighted image). (B) Brain abscess in the left parietal lobe, observed via brain MRI (T2 weighted image). (C) multiloculated left pleural effusion, observed via chest computed tomography (CT). (D) Right pleural effusion with atelectasis in the right middle and lower lobes, observed via chest CT.

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### Declaration of Interest:

The authors have no potential conflicts of interest to declare.

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