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Case Report

First case of *Nocardia nova* spinal abscess in an immunocompetent patient

Seung Bok Hong[a], *Kyudong Han*[b], *Bo Ra Son*[c], *Kyeong Seob Shin*[c*], *Byeong Cheol Rim*[d]

[a]*Department of Clinical Laboratory Science, Juseong University, South Korea*
[b]*Department of Microbiology and Institute of Basic Science, Dankook University, South Korea*
[c]*Department of Laboratory Medicine, College of Medicine, Chungbuk National University, South Korea*
[d]*Department of Neurosurgery, College of Medicine, Chungbuk National University, South Korea*

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ABSTRACT

Nocardia are a group of aerobic actinomycetes that are filamentous gram-positive, weakly acid-fast, and cause opportunistic infection in immunocompromised patients. Primary *Nocardia* infection mostly involves lung, skin and less commonly, the central nervous system (CNS). Among *Nocardia* CNS infections, spinal infection is extremely rare. We describe the first case of a spinal abscess caused by *Nocardia nova* in an immunocompetent patient who experienced a penetrating facial injury six months earlier. *Nocardia* species were isolated from intradural spinal abscesses and identified by 16S rRNA, *hsp65* and *secA1* sequence analyses. Surgical excision and treatment with amikacin, cefotaxime, and oral erythromycin was successful.

Introduction

Nocardia species are a group of aerobic actinomycetes that are characteristically filamentous, branching, Gram-positive and modified acid-fast bacillus.[1] Although *Nocardia* normally exist as soil saprophytes, increasing numbers of infections have been observed in immunocompromised individuals. Tsukamura first described *N. nova* in 1982 as a separate species with distinct features from the *N. asteroides* complex and *N. farcinica*.[2] In 1990, Yano et al. reported that *N. nova* had different DNA homology with *N. asteroides* (39% homology) and *N. farcinica* (20% homology).[3]

It is an unusual pathogen that commonly causes pneumonia[4] or cutaneous abscess,[5] and rarely, osteomyelitis[6] or sinusitis.[7] The involvement of the central nervous system (CNS) by *Nocardia* occurs in up to 20% of the patients, but solitary spinal abscess due to *Nocardia* is extremely rare.[8-10] *Nocardia* spinal abscesses are typically present as intra-medullary or epidural lesions, usually caused by *N. asteroides*.[8-10]

We were able to find only three cases of *N. nova* CNS infection in the English language literature search (PubMed), including two cases of brain abscess and one of spondylodiscitis.[11-13] All three patients were immunocompromised, as a result of either steroid administration, renal transplantation, or human immunodeficiency virus (HIV) infection. To our knowledge, however, no case of spinal abscess caused by *N. nova* has

* *Corresponding author at:* Department of Laboratory Medicine, College of Medicine Chungbuk National University, 52 Naesudong-ro, Heungduk-gu, Cheongju, Chungbuk 361-763, South Korea
E-mail address: ksshin@chungbuk.ac.kr (Kyeong Seob Shin)

ever been reported. Additionally, this was a case of primary nocardiosis without a pulmonary infection or cutaneous lesions, and it lacked predisposing factors, including immunosuppressive therapy, hematologic malignancy, and transplantation.

Case presentation

A 54-year-old female visited the emergency department because of progressive bilateral leg weakness and uncontrollable bowel movements. Upon arrival, she had complete paraplegia in both legs without fever. Six months earlier, she had experienced a penetrating injury in the left orbital area with continuous cerebrospinal fluid (CSF) rhinorrhea. At that time, she was treated with antimicrobials for three months following surgical intervention.

At this visit a laboratory evaluation showed a slightly elevated C-reactive protein level of 14.5 mg/L. The patient's white blood cell (WBC) count was 7,200/mm^3 with 65% polymorphs. Her CSF examination contained 4,046 mg/dL protein, 70 mg/dL glucose, and 80 WBC/mm^3. A CSF smear was negative on both Gram and acid-fast bacillus (AFB) staining. Bacterial cultures of the CSF were also negative. Magnetic resonance imaging with gadobutrol contrast revealed a diffusely increased intra-medullary signal intensity from T7 to the cauda equina, which suggested arachnoiditis, and multifocal expansion with rim enhancement of the spinal cord at T11-L1 and L3, suggesting multiple abscesses (Fig. 1).

The patient underwent a T11-L1 laminectomy, and spinal intradural and extra-medullary abscesses were removed from the T11-L1 level and cultured. The pus contained long filamentous organisms, as revealed by Gram staining. After three days of culture, small, wrinkled white colonies composed of Gram-positive cells arranged in branching filaments were observed. The microorganism was negative for AFB, but positive for modified AFB, suggesting a *Nocardia* species.

Accurate identification was made by 16s rRNA, *hsp65*, and *secA1* sequencing[14] (Table 1). The 16S rRNA gene sequence (921 bp) from the isolate CBU 09/875 showed 100% similarity with those of several *N. nova* strains including EU74135, DSM40806 and IFM0261. However, the strain showed similarity values of 99.56% with *N. pseudosporangifera* Z37136 and, 99.02% with *N. jiangxiensis* DQ840027, which are non-pathogenic to humans. The *hsp65* sequence (409 bp) from the CBU 09/875 isolate showed the greatest similarity (99.51%) to three *Nocardia* species (*N. nova* AY75766527, *N. veterana* AY7576538 and *N. africana* AY756512). The *secA1* sequence (438 bp) showed the greatest similarity (99.54%) to *N. nova* GU179114 and *N. aobensis* EU178744.

Based on our 16S rRNA, *hsp65* and *secA1* sequence analyses, we concluded that the CBU 09-875 isolate was *N. nova*. The 16S rRNA gene sequence of this isolate was assigned to the GenBank nucleotide sequence database

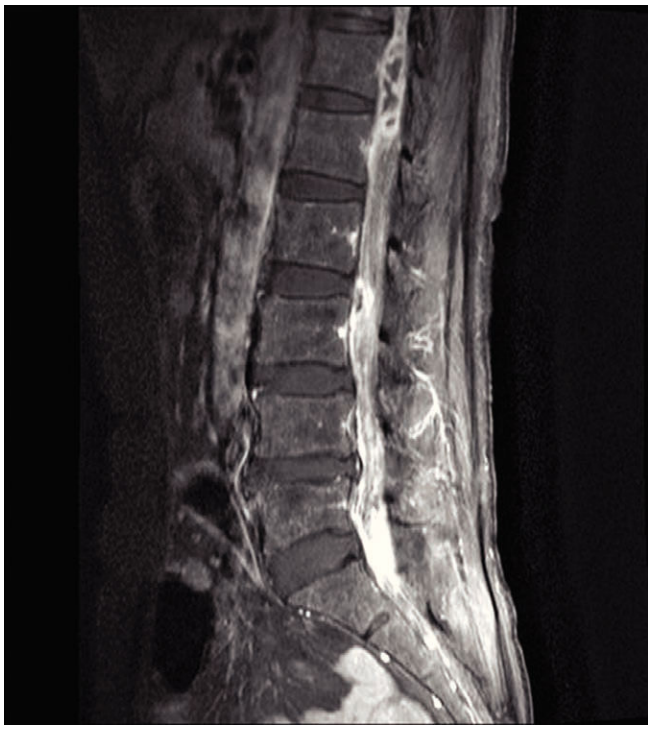

Fig. 1 - Sagittal T1-weighted MRI of the lumbosacral spine with contrast enhancement. Multifocal enhancing lesions are visible at levels T11-L1 and L3. Diffusely increased intramedullary signal intensity is evident from the lower thoracic level to the cauda equina.

Table 1 - PCR primers and DNA amplification conditions for 16S rRNA, *secA1* and *hsp65*

Target	Primer (5' to 3')	PCR cycling conditions	Reference
16S rRNA	27F: AGA GTT TGA TCM TGG CTC AG	5min 95°C	14
	1492R: TAC GGY TAC CTT GTT ACG ACT T	35X (45s 94°C, 1min 55°C, 1min 72°C), 10min 72°C	
secA1	F: GCG ACG CCG AGT GGA TGG	30s 98°C,	17
	R: TTG GCC TTG ATG GCG TTG TC	35X (5s 98°C, 5s 67°C, 20s 72°C), 1min 72°C	
hsp65	F: ACC AAC GAT GGT GTG TCC AT	30s 98°C,	17
	R: CTT GTC GAA CCG CAT ACC CT	35X (5s 98°C, 5s 54°C, 20s 72°C), 1min 72°C	

under accession number HM584914. The phylogenic relationships of isolate CBU 09-875 with other related *Nocardia* strains based on 16S rRNA gene sequences are shown in Fig. 2.

The patient was treated empirically with amikacin, cefotaxime and vancomycin. After identification of the *Nocardia* species and antimicrobial susceptibility testing by disk diffusion, the regimen was changed to amikacin and cefotaxime for three months. The isolate was found to be sensitive to ampicillin, erythromycin, amikacin, imipenem, cefotaxime and trimethoprim-sulfamethoxazole (TMP-SXT) but resistant to penicillin, amoxicillin/clavulanic acid and tobramycin by E-test, which is compatible with the typical antibiotic susceptibility pattern of *N. nova*.[15]

The patient had a favorable clinical response. Treatment was continued on an outpatient basis with erythromycin; the patient completed a year of treatment. Five months postoperatively, the patient had recovered sufficiently to walk with an aid, and had better bowel control. After one year, she was able to walk without support.

Discussion

Biochemical methods for identifying *Nocardia* species are limited in their ability to differentiate these organisms. The application of molecular methods, including sequence analysis of the *Nocardia* 16S rRNA gene, has greatly expanded the knowledge of the spectrum of pathogenic *Nocardia* species.[16] Meanwhile, because 16S rRNA gene sequencing alone is sometimes insufficient for the identification of *Nocardia* species, the sequencing of other conserved regions, such as *hsp65* and *secA1*, can serve as adjuncts to 16S rRNA sequencing.[17,18]

In this case, the 16S rRNA gene sequence of CBU 09/875 showed complete identity with *N. nova* and strong similarity (> 99%) with two other *Nocardia* species, which have not yet been reported to be responsible for human infections. To improve the differentiation, *hsp65* and *secA1* were sequenced, and showed the greatest similarity with *N. nova*, *N. veterana* and *N. africana* among the species pathogenic to humans. Based on these data, CBU 09/875 was identified as *N. nova*; this was subsequently confirmed by biochemical testing, including a positive two-week arylsulfatase assay and an inability to degrade casein, xanthine, tyrosine and hypoxanthine (data not shown).

Nocardiosis is often difficult to treat; the choice of therapy should therefore be guided by susceptibility testing. Treatment with a combination of antibiotics is necessary to avoid both the recurrence of infection and drug resistance. Additionally, prolonged antibiotic therapy from six months to a year or longer is required, due to the slow replication rate of the

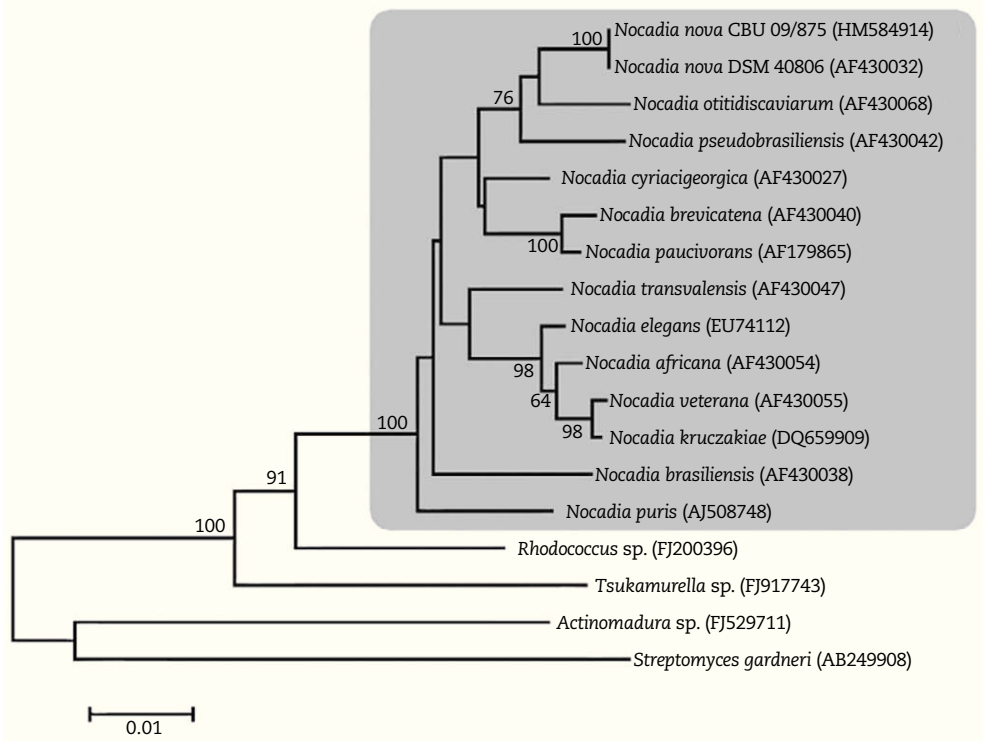

Fig. 2 - Phylogenic tree of the current isolate (CBU 09/875) and closely related aerobic actinomycetes, including other *Nocardia* species. This neighbor-joining tree was constructed using 16S rRNA gene sequences, based on the Kimura two-parameter distance. Bootstrap values (%) are shown near their corresponding branches; '0.1' indicates 0.1 nucleotide substitutions per site.

organism.[19] A three-drug regimen comprising TMP-SXT, amikacin, and either ceftriaxone or imipenem has been used successfully.[19] Patients with abscesses may require surgical drainage to ensure the adequate penetration of antibiotics and clearance of the bacteria.

N. nova is characterized by susceptibility to erythromycin, clarithromycin, amoxicillin, and linezolid, and by resistance to amoxicillin/clavulanic acid. The case we describe here was successfully treated with a combination of amikacin and cefotaxime, following by oral erythromycin based on antibiotic susceptibility testing.

Human infections by *Nocardia* result from trauma-related introduction of the organism, as well as from inhalation, with the resulting establishment of a pulmonary focus.[20] In the present case, the patient had a history of an orbital penetrating injury with CSF rhinorrhea six months prior, and since then she had undergone surgical intervention and antibiotic treatment for three months. Additionally, she lacked the predisposing factors for *Nocardia* infection, including immunosuppressive therapy, hematologic malignancy, transplantation, etc. Consequently, in this case, the spinal abscess by *N. nova* likely resulted from the previous orbital injury rather than the surgical intervention at that time.

This is the first reported case of a spinal abscess without extra-neural infection caused by *N. nova* in an immunocompetent host after a skull-penetrating injury. Rapid and accurate identification of the *Nocardia* species by various molecular methods including 16S rRNA, *hsp65* and *secA1* sequence analyses will assist in the provision of appropriate treatments to patients infected with *Nocardia*, because the antimicrobial susceptibility revealed the patterns that are typical among *Nocardia* species.

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Conflict of interest

All authors declare to have no conflict of interest.

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