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# *Nocardia kroppenstedtii*: a rare pathogen isolated from the spinal vertebral abscess of a patient on long-term immunosuppressive therapy

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**Abstract. – OBJECTIVE: *Nocardia kroppenstedtii* was isolated from the spinal vertebral abscess of a 78-year-old patient presenting with mid-thoracic pain and bilateral lower limb weakness and numbness. The patient was on long-term immunosuppressive therapy with steroids for underlying autoimmune hemolytic anemia. Investigations showed a T5 pathological fracture and vertebra plana with the erosion of the superior and inferior endplates. There was evidence of paraspinal collection from the T4-T6 vertebrae with an extension into the spinal canal. Analysis of *Nocardia* 16S rRNA (99.9%, 1395/1396 nt) and *secA1* gene (99.5%, 429/431 nt) fragments showed the highest sequence similarity with *Nocardia kroppenstedtii* type strain (DQ157924), and next with *Nocardia farcinica* (Z36936). The patient was treated with intravenous carbapenem and oral trimethoprim-sulfamethoxazole for four weeks, followed by another six months of oral trimethoprim-sulfamethoxazole. Despite the improvement of neurological deficits, the patient required assistive devices to ambulate at discharge. This study reports the first isolation of *N. kroppenstedtii* from the spinal vertebral abscess of a patient from Asia. Infections caused by *N. kroppenstedtii* may be underdiagnosed as the bacterium can be misidentified as *N. farcinica* in the absence of molecular tests in the clinical laboratory.**

*Key Words:*
*Nocardia kroppenstedtii, Nocardia farcinica,* Spinal infection, 16SrDNA, *secA1*

## Introduction

*Nocardia* spp. are Gram-positive, saprophytic, aerobic actinomycetes, which appear as partial acid-fast, beaded, branching filaments[1]. Of 92 *Nocardia* spp. recognized to date (http://www.bacterio.net/nocardia.html), more than half (54) have been reported to cause human infections[2]. The bacteria are found ubiquitous in water, soil, dust, decaying vegetation, and organic matters. Transmission through inhalation or percutaneous inoculation from environmental sources, especially in patients with immunosuppression, can lead to life-threatening multisystemic infections[3]. As antibiotic resistance has been associated with certain *Nocardia* spp., speciation and antibiotic susceptibility testing of *Nocardia* are essential to guide antimicrobial therapy[4].

Gene sequencing has been identified as a gold standard for speciation of *Nocardia* isolates[4]. Since *Nocardia* 16S rRNA genes are well conserved, *secA1*, a housekeeping gene encoding essential protein SecA1, has been used as a target for discriminating *Nocardia* spp.[5]. The species status of *Nocardia* isolates can be established when *secA1* gene exhibits >99.0% similarity with the type strain.

### Case Report

A 78-year-old female patient presented to our hospital with bilateral lower limb weakness and numbness for two weeks and mid-thoracic pain for three months. She was on long-term immunosuppressive therapy with steroids for underlying autoimmune hemolytic anemia. Plain radiographs showed a T5 pathological fracture. Magnetic resonance imaging (MRI) revealed vertebra plana with the erosion of the superior and inferior endplates. There was evidence of

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paraspinal collection from T4-T6 vertebrae with extension into the spinal canal, resulting in spinal canal stenosis and spinal cord compression. Features suggestive of subligamentous spread were also observed. Elevation of C-reactive protein (6 mg/dL) and high white cell counts ($14.9x10^9$ /L) with neutrophil predominance was noted. As her neurological deficit deteriorated two days after admission, emergency posterior decompression, debridement, drainage of the abscess were performed. She was given empirically intravenous cloxacillin after surgery. The pus sample drained from the T5 vertebral body grew tiny whitish colonies on blood agar. The isolate (labelled as N1) was a Gram-positive filamentous bacillus, which was partially acid-fast upon staining using the modified Ziehl-Neelsen method. It was suspected to be a *Nocardia* species and was subjected to further speciation using 16S rRNA gene sequencing approach.

Upon learning of the isolation of *Nocardia* sp., the patient's empirical antibiotic therapy was changed to intravenous carbapenem and oral trimethoprim-sulfamethoxazole at 15 mg/kg/day for four weeks. A repeat MRI scan performed at the end of the intravenous therapy showed resolution of subligamentous collection and improvement in the paraspinal collection. At this point, as her inflammatory markers had normalized, treatment was continued with only oral trimethoprim-sulfamethoxazole at 15 mg/kg/day for six months. She also received ongoing physiotherapy rehabilitation. Her back pain resolved at discharge; however, she still required assistive ambulation devices as her neurological deficit had not improved to normal grade.

*Nocardia* sp. N1 grew as whitish and greyish colonies on blood agar after 24-48 hours of incubation. Upon prolonged incubation, dry, and pale yellowish colonies appeared at the peripheries of the streaking line. Bacterial DNA was extracted from both white and grey colonies using a boiling method. Multiple primers (27F, 780R, 529F, 1099R, 925F, and 1491R) were used for amplification of the 16S rRNA gene[6]. Two primers, 5′GACAGYGAGTGGATGGGYCGSGTGCACCG3′ and 5′GCGGACGATG TAGTCCTTGTC3′, modified from Conville et al[5] were used for *secA1* gene amplification. Sequence determination of the amplified fragments was performed in an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA), using forward and reverse primers of each PCR assay. Sequence homology searches were performed using the Basic Local Alignment Search Tool program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A dendrogram was constructed based on *secA1* sequences of various *Nocardia* type strains, using the neighbor-joining method (Tamura-Nei model) and bootstrap analysis with 1,000 resamplings[7].

The recommended sensitivity testing for *Nocardia* sp. is broth microdilution method[8]; however, as this method was not available in our laboratory, sensitivity testing was performed using E test (bioMérieux-SA, Marcy-l'Etoile, France) against imipenem, ceftriaxone, and trimethoprim-sulfamethoxazole.

Amplified 16S rRNA gene fragments from white and grey colonies of *Nocardia* sp. N1 showed matching nucleotide sequences, suggesting that the colonies were morpho-variants. The nearly full length 16S rRNA gene sequence (Genbank accession no: MT261824) demonstrated the highest sequence similarity (99.92%, 1395/1396 nt) with *Nocardia kroppenstedtii* (Genbank accession no: DQ157924) and *Nocardia farcinica* (Genbank accession no: Z36936, 99.85%, 1394/1396 nt). Sequence comparison of *Nocardia secA1* fragment (Genbank accession no: MT271614) displayed the highest sequence similarity with *N. kroppenstedtii* (Genbank accession no: KX925843, 429/431 nt, 99.5%), followed by *N. farcinica* (Genbank accession no: DQ360274, 428/431 nt, 99.3%).

Figure 1 shows the clustering of *N. farcinica, N. kroppenstedtii* and *Nocardia* sp. N1 in a separate branch of the *secA1* phylogenetic tree with a strong bootstrap value (100%). The phylogenetic analysis reveals a close genetic relationship amongst *N. farcinica, N. kroppenstedtii* and *Nocardia* sp. N1. As *Nocardia* sp. N1 exhibits the highest sequence similarity in both 16S rRNA and *secA1* gene, it is tentatively referred to as *N. kroppenstedtii* in this study. E tests showed the minimum inhibitory concentration (MIC) values of *Nocardia* sp. N1 against trimethoprim-sulfamethoxazole and imipenem were 1.0 μg/ml and 0.75 μg/ml, respectively, suggesting susceptibility to both drugs[8]. In contrast, a high MIC was noted against ceftriaxone (≥32 μg/ml).

## Discussion

*Nocardia kroppenstedtii* is a novel pathogen first isolated from the bronchial lavage of a lung transplant patient with pulmonary infection[9]. Infection caused by this organism was later report-

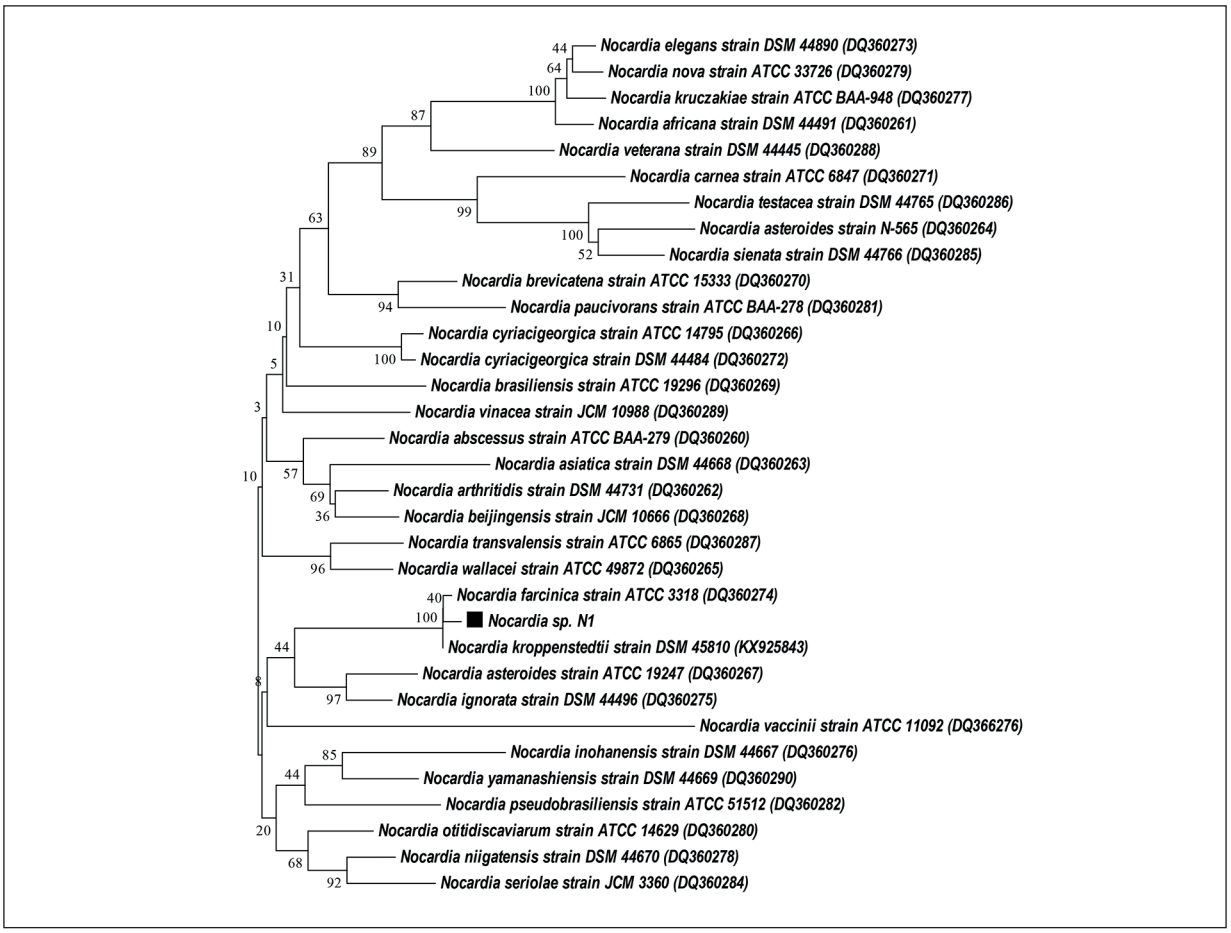


**Figure 1.** A dendrogram constructed based on *Nocardia secA1* gene sequences. *Nocardia* sp. N1 (■) is clustered with *N. farcinica* and *N. kroppenstedtii* in a distinct branch with 100% boot strapping value in the dendrogram.

ed in a 72-year-old Caucasian immunosuppressed patient with disseminated nocardiosis, brain abscess and infective endocarditis[10]. In a recent USA study, *N. kroppenstedtii* (besides *N. farcinica*) was isolated from blood cultures of a 59-year-old Caucasian woman with a history of multiple malignancies. She was diagnosed with endogenous endophthalmitis; additionally, her brain MRI also showed lesions consistent with brain abscess[11].

In contrast, *N. farcinica* infection is reported more frequently in clinical cases. The organism has a propensity to disseminate hematogenously from the primary source of infection to the brain, kidneys, joints, bones, and eyes of immunocompromised patients[12]. *N. farcinica* is a rare cause of spinal infection[13]. To the best of our knowledge, only two cases of *N. farcinica* spinal infection have been reported so far. Spinal osteomyelitis due to *N. farcinica* was first described in a 54-year-old man with a history of alcohol abuse and chronic liver disease[14]. In the second case, *N. farcinica* was isolated from the spinal epidural abscess of a 50-year-old man with diabetes in China[15].

With the increasing use of immunosuppressive therapy, the occurrence of *Nocardia* is likely to rise among immunosuppressed patients. As nocardial infection is rarely reported, infections caused by *N. kroppenstedtii* are probably underdiagnosed. Additionally, the bacterium may be misidentified as *N. farcinica* due to a lack of discriminating tests in the clinical laboratory. Currently, identification of *N. kroppenstedtii* by MALDI-TOF mass spectrometry is not possible due to the unavailability of reference spectra in the database. Specific tests for *Nocardia* systematics and DNA-DNA relatedness study are recommended for confirmation of *N. kroppenstedtii*[9]; however, these tests are laborious, costly and not available in clinical laboratories.

## Conclusions

Identification of *N. kroppenstedtii*, an emerging opportunistic pathogen, remains a challenge in the absence of specific tests in the clinical microbiology laboratory. This study describes the first isolation of *N. kroppenstedtii* from the spinal vertebral abscess of a patient in Asia. Infections caused by *N. kroppenstedtii* may be underdiagnosed as the bacterium can be misidentified as *N. farcinica* in the absence of molecular tests in the clinical laboratory.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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