

First Report of the Isolation of *Nocardia thailandica* from the Bronchoalveolar Lavage of a Patient in Iran

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Abstract

Nocardiae are Gram-positive, filamentous, aerobic, relatively slow-growing, and weakly acid-fast bacteria which cause nocardiosis in humans. We describe a 53-year-old patient with chronic bronchitis referred to Al-Zahra Hospital, Isfahan. A bronchial washing sample was taken from the patient. A *Nocardia*-like microorganism was detected in microscopic evaluation. Based on the phenotypic and 16S rRNA gene sequencing, the isolate was identified as *Nocardia thailandica*. The patient was treated with trimethoprim-sulfamethoxazole and linezolid. This is the first report of the isolation of *Nocardia thailandica* from Iran.

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Introduction

Nocardiae are Gram-positive, aerobic, slow-growing, and relatively acid-fast bacteria which live as saprophytes in environmental sources such as water, soil, dust, animal excreta, and decaying plants. This group of bacteria can enter the human body through inhalation and skin injuries, creating nocardiosis infection.¹ *Nocardia* infections can be hosts for immune system disorders, and they can even involve healthy individuals.²

More than 30 different species of *Nocardia* have so far been isolated from clinical samples, among which *Nocardia thailandica* (*N. thailandica*) was first introduced by Kageyama and colleagues from the purulent secretions of a Thai patient's abscess. According to the existing reports, *N. thailandica* is isolated from respiratory and eye infections.¹⁻³

Given the scarcity of reports in the existing literature on the isolation of *N. thailandica*, we sought to conduct the present case study on respiratory infections caused by *N. thailandica*.

Case Presentation

A 53-year-old Iranian man with chronic bronchitis was admitted to Al-Zahra Hospital, Isfahan, because of mild fever, weight loss, nonproductive cough, anorexia, and chronic chest pain. Diffuse nodular infiltrates and enlargement of blood vessels were observed in the radiography images of the patient's chest (figure 1). There was no apparent evidence of immunodeficiency or HIV infection. Microscopic examination (acid-fast staining) of 3 sputum samples did not reveal acid-fast bacteria. Additionally, the tuberculin

What's Known

- *Nocardia thailandica* is an emerging pathogen.

What's New

- This manuscript is the first report of the isolation of *Nocardia thailandica* from Iran.
- Isolation of this microorganism is important in epidemiological studies.



Figure 1: Chest X-ray from the patient shows nodular opacities and small irregular opacities in the lung.



Figure 2: Kinyoun staining of the *Nocardia thailandica* shows characteristic partially acid-fast branching rods.

test on the patient proved negative. Because the patient's fever and chest pain remained unchanged, the treating physician requested a bronchoalveolar lavage sample. The sample was incubated at 37 °C in a blood agar medium containing 5% sheep blood, chocolate agar, and brain heart infusion (BHI) agar. White and dry colonies emitting soil odor were observed in the chocolate and BHI mediums after 3 days. A *Nocardia*-like organism was detected in the direct microscopic evaluation of the patient's bronchoalveolar lavage (Gram-positive and weakly acid-fast with branching filamentous rods) (figure 2). This isolate was subjected to identification by resistance to lysozyme broth and conventional phenotypic tests.¹

Primarily, the bacterial genome was extracted via the boiling method for molecular investigations. Then, 27-F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492-R (5'-GGTTACCTTGTTACGACTT-3') primers were used to reproduce the 16S rRNA gene as recommended by Morelli et al.⁴ Sequencing was performed by Bioneer Corporation (South Korea), and the sequence data received were aligned using CLC Main Workbench 5 software with the existing sequences of *Nocardia* retrieved from the GenBank in the National Center for Biotechnology Information (NCBI) website database. The resultant data were thereafter analyzed using the Basic Local Alignment Search Tool (BLAST) program in GenBank. According to the results, the considered microorganism was identified as *N. thailandica* and the studied isolation sequences were registered in the NCBI under accession No. KC577173.1. The partial 16S rRNA gene sequences (1387 bp) of the isolate revealed 99% similarities with those of the *N. thailandica* strain DSM 44808.

The disk diffusion method was utilized to test the determination of the antimicrobial susceptibility test for the considered isolation. The considered microorganism was sensitive to trimethoprim-sulfamethoxazole, amikacin, and linezolid, while it was reported to be resistant to penicillin-G and ciprofloxacin. The patient was treated with trimethoprim-sulfamethoxazole (480 mg) and linezolid (600 mg) for 6 months and his symptoms eventually disappeared completely. Informed consent to report this case was obtained from the patient.

Discussion

Nocardia spp. are Gram-positive, weakly acid-fast, nonspore-forming, filamentous, branching, obligatory aerobic, and relatively slow-growing bacteria which often form branched hyphae in both tissue and culture. The hyphae are easily disrupted into rods and cocci elements. *Nocardia* spp. are found extensively around the world as a saprophytic component of the soil, water, dust, and air. *Nocardia* was first isolated by Edmond Nocard in 1888 from a case of bovine farcy. A year after, an Italian researcher, Trevisan, characterized the organism and named it *N. farcinica*.¹

The prevalence of *Nocardia* infections has risen in recent years. This phenomenon can be the result of factors such as increasing numbers of patients with immunodeficiency and the use of advanced molecular methods.^{5,1} The detection of clinically significant *Nocardia* spp. is important for the definitive diagnosis, prediction of antimicrobial susceptibility, epidemiological purposes, and effective treatment.^{1,3} Owing to the similarity between *Nocardiae* and fungi and the non-exclusivity of clinical manifestations as well

as radiologic findings in nocardiosis infections, *Nocardia* infections can be mistaken with fungal infections and mycobacteria.^{5,6} Molecular methods can identify *Nocardia* infections with acceptable precision and exclusivity. For instance, finding the sequence of the 16S rRNA gene is one of the best alternatives for determining and comparing *Nocardia* spp.^{7,8} Corticosteroid and immunosuppression treatment of a patient with chronic bronchitis is one of the most important risk factors in respiratory nocardiosis infections.⁹

Shahraki et al.⁸ collected 127 clinical isolates of *Nocardia* spp. from patients living in 5 different Iranian provinces and found that *N. cyriacigeorgica* and *N. asteroides* were the most common species isolated from Iranian patients with nocardiosis. They also reported that trimethoprim-sulfamethoxazole and linezolid were respectively the first- and second-line antibiotics against nocardiosis. The most common manifestation of *Nocardia* infections is the pulmonary form, and the first isolate of *N. cyriacigeorgica* was from a patient affected by chronic bronchitis.⁶ Canterino et al.¹⁰ (2015) published a case study on *N. thailandica* respiratory infection in a patient receiving a transplantation. Whereas the patient in their study was immunocompromised, we isolated this species from an older man. Thus, *N. thailandica* is an opportunistic pathogen that has emerged in recent years. Reddy et al.¹¹ reported a single case of keratitis caused by *N. thailandica*. There is a dearth of data on the isolation of *N. thailandica*. In the present study, the microorganism isolated from the patient suffering from chronic bronchitis was evaluated via phenotypic and molecular tests and identified as *N. thailandica*. Based on DNA sequences, *N. thailandica* is closely related to *N. asteroides*, *N. neocaledoniensis*, and *N. caishijiensis*. Therefore, the identification of this species requires trained technicians.¹⁰ In Iran, Shahraki et al.⁶ isolated 11 different *Nocardia* spp. from 46 patients with nocardiosis and reported that *N. asteroides* and *N. veterana* were respectively the most and least frequent *Nocardia* spp. isolated from the patients. *Nocardia* identification at the species level is vitally important for appropriate treatment, production of secondary metabolisms especially antibiotics, and epidemiologic and geographic studies.¹²

Our literature review yielded precious few clinical reports about the isolation of *N. thailandica* around the globe. Because this species is susceptible to the majority of antimicrobials for other *Nocardia* spp.,^{8,10} the isolation and identification of this species is important for epidemiological studies into

the distribution of *Nocardia* spp. in the world. *N. thailandica* IFM 10145^T was first isolated as a novel species in 2004 from a wound infection³ and has also been reported from pulmonary and keratitis infections. *N. thailandica* infections can be treated with trimethoprim-sulfamethoxazole, imipenem, amikacin, tobramycin, and the third generation of cephalosporins.^{3,10}

Our report may help clarify the taxonomy of *N. thailandica* and its role as a member of the genus *Nocardia*, which can cause nocardiosis in men, specially pulmonary nocardiosis.

Conclusion

N. thailandica is a possible emerging pathogen within the *Nocardia* genus and is often accompanied by pulmonary infections. Molecular methods can identify *Nocardia* spp. faster and better than conventional methods. Due to the similarity between *N. thailandica* and *N. asteroides*, 16S rRNA gene sequencing is the best method for the rapid and accurate identification and differentiation of *Nocardia* spp.

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Conflict of Interest: None declared.

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