Case report

Nocardia arthritidis as a cause of disseminated nocardiosis in a patient with chronic lymphocytic leukemia

Amity L. Roberts*, Rebecca M. Davidson, Alison G. Freifeld, Peter C. Iwen

*Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States
bDepartment of Pathology, University of Nebraska Medical Center, Omaha, NE, United States
cDepartment of Pathology, Anatomy and Cell Biology, Sidney Kimmel Medical College at Thomas Jefferson University, 117 N 11th Street, PA 207, Philadelphia, PA 19107, United States
dCenter for Genes, Environment and Health, National Jewish Health, Denver, CO, United States

Abstract

A case of disseminated nocardiosis caused by Nocardia arthritidis in an immunocompromised patient with a history of chronic lymphocytic leukemia and rheumatoid arthritis is presented. This report highlights the use for multilocus sequence typing (MLST) in addition to single gene molecular sequencing to identify rare Nocardia species.

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Case report

A 71-year-old Caucasian female with a history of chronic lymphocytic leukemia (CLL) and rheumatoid arthritis (RA) presented to her primary care physician with a 2 month history of worsening cough and dyspnea without fevers, chills or sinus congestion. She was maintained on a low dose of prednisone (homeopathic not treatment dose), at 5 mg daily, for treatment of RA. Four years prior to presentation, she was treated with chlorambucil for 6 months followed by IV immunoglobulin for 9 months for CLL.

At presentation, the patient underwent a computed tomography (CT) scan which showed a 4.5 cm non-calcified solid mass in the superior segment of the right lower lobe (RLL) followed by an ultrasound (US) guided needle biopsy. A Gram-stain of the biopsy showed gram-positive branching, beaded filamentous bacilli; however, culture was not performed at this time. The histology (performed by an outside institution) was originally negative for both bacterial and fungal elements. The patient was subsequently treated empirically with 5 weeks of oral levofloxacin (750 mg, once daily) followed by 2 weeks of oral azithromycin (500 mg daily) without improvement. Following a continued complaint of progressive breathlessness, exacerbated by activity, the patient was referred to The Nebraska Medical Center (TNMC) for further evaluation as an outpatient.

When seen in the TNMC outpatient clinic, the patient appeared well and her physical examination was normal. The absolute lymphocyte count was 1300 µL⁻¹ (normal 700–3900 µL⁻¹). Repeat CT scan demonstrated enlargement of the RLL mass with new cavitation. A CT-guided fine-needle biopsy of the RLL mass was performed 5 days prior to inpatient admission. Histological exam of the tissue revealed organizing acute and chronic inflammation which was reported negative for bacteria by Fite-Faraco modified acid fast (FITE) stain and negative for bacterial and for fungal elements by the Gomori methenamine-silver (GMS) stain. Five days later the patient developed fever, chills and dyspnea and was admitted to TNMC (day 0, post-admission). The RLL biopsy tissue subsequently became culture positive for a Nocardia species 8 days after culture on Sabouraud dextrose agar (SAB). The FITE stain of the TNMC RLL fine-needle biopsy was subsequently re-reviewed at 1000× magnification and determined to be positive for rare long thin branching filamentous bacilli that were modified acid-fast negative. Consequently the histology stain report was amended to state, “morphologically consistent with nocardiosis.” The re-reviewed GMS-stained biopsy remained negative for bacterial and fungal elements.

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Empiric antibacterial and antifungal agents were discontinued and high-dose oral trimethoprim-sulfamethoxazole (TMP-STX) (2 double-strength tablets (800 mg/160 mg), twice a day) was initiated. Although no neurological symptoms were present and the neurologic exam was normal, a magnetic resonance imaging (MRI) of the brain was performed due to the known propensity for Nocardia species to disseminate to the central nervous system (CNS) in immunocompromised individuals. The MRI detected multiple intracerebral multifocal ring enhancing lesions, indicative of nocardiosis (Fig. 1A, B). On day 4, PA, the patient developed a nodular lesion on the lower right leg. Biopsy of this leg lesion and histologic staining again showed the presence of modified acid-fast stain negative branching filamentous bacteria, subsequently identified in culture as Nocardia species. The patient was treated with a course of oral TMP-STX for a planned duration of 12 months, in conjunction with IV ceftriaxone (2 g every 12 h) for the initial 3 months. Serum sulfamethoxazole levels were maintained at 12–16 mg/dL range over the duration of therapy.

A marked improvement of pulmonary symptoms and a decrease in the size of the brain lesions as observed by MRI after 12 weeks of combination therapy was noted. The patient continued to improve and never developed neurologic symptoms. MRI of the brain after one year of treatment with high-dose oral TMP-STX showed resolution of the abscesses and the drug was discontinued. Two months later she had a sudden syncope episode and although no changes were seen on repeat brain MRI, oral TMP-SMX was re-instituted for chronic maintenance therapy.

Materials and methods

To determine the Nocardia species and to obtain the antimicrobial susceptibility testing (AST) profile, two isolates (SQ-14–947 and SQ-14–948) were submitted to an outside reference laboratory for testing (ARUP Laboratories, Salt Lake City, UT). Sequence analysis of the partial 16S rRNA target identified the isolates as Nocardia abscessus complex/Nocardia gankensis. The AST minimum inhibitory concentration (MIC (µg/mL)) results for both case isolates were: amoxicillin/clavulanate ≥128/64 (Resistant (RR)); ceftriaxone ≤4 (Susceptible (SS)); ciprofloxacin ≥8 (RR); clarithromycin 4 (Intermediate); imipenem ≤4 (SS); tobramycin ≤1 (SS); and trimethoprim/sulfamethoxazole ≤0.25/4.8 (SS). The AST profiles and the corresponding drug patterns were inconsistent with the Type I drug susceptibility pattern for N. abscessus complex [1] and neither a drug pattern nor antimicrobial susceptibilities had been described for N. gankensis [2,3]. Interestingly, N. gankensis has yet to be reported in association with human disease, with the original isolates being discovered in soil removed from the Gamka River in Western Cape Province, South Africa [3]. Based on the AST pattern, additional analyses were pursued to confirm the species level identification. Moreover, multiple reports have shown that the partial 16S rRNA gene sequence could not differentiate between Nocardia exalbida and Nocardia gankensis [1,4–8].

To further evaluate the species identification, the complete 16S rRNA gene as well as the secA gene and hsp65 gene were sequenced and analyzed by the Mycobacterial/Nocardia Reference Research Lab at the University of Texas Health Science Center at Tyler (UTHSC, CLIA regulated). Each gene was sequenced and analyzed per the UTHSCST standard protocol [5,9,10]. The sequence analysis results showed that the complete 16S rRNA gene resulted in 99.9% match (1391/1392 bp) to N. arthritidis type strain, 99.5% match (1406/1413 bp) to N. araoensis type strain, and 99.0% match (1399/1413) to N. exalbida type strains for both isolates. All of these species belong to or are closely associated with N. abscessus complex. The CLSI recommends that ≥99.6% identity occur to confirm for genus and the species identification [10]. SecA amino acid analysis of secA1 gene sequencing provided a 100% match to N. arthritidis and N. exalbida type strains and 99.36% match to N. araoensis type strain giving a final identification of Nocardia arthritidis/exalbida/araoensis. For Nocardia, secA1 has been the preferred gene for sequencing, and for 0–1 amino acid mis-match corresponding to ≥99% identity consider reporting the confirmed genus and species [5]. Finally, hsp65 gene sequencing resulted in a 98.8% match (417/422 bp) to N. arthritidis type strain, 98.3% match (393/400 bp) to N. exalbida type strain and 97.4% match (411/422 bp) to N. araoensis. There are currently no CLSI guidelines for the interpretation of hsp65 gene sequences in Nocardia species.

To improve on the resolution of the single gene sequencing results, a multi locus strain typing (MLST) approach was performed for all three gene targets (16S rRNA, hsp65 and secA). Gene sequence data for the 3 loci were extracted for 10 diverse Nocardia species (Table 1) from the prokaryotic genome database at the National Center for Biotechnology Information (NCBI), and sequences were concatenated for each species. Then, neighbor joining phylogenetic analyses [11] with 100 replicate searches were performed for each gene separately and the concatenated sequences (MLST) for all 12 strains using the SeaView software package [27]. The results following analysis showed that for Phylogenies: 1) for the individual gene analyses, 2 of the 3 genes (16S rRNA and hsp65) suggested that SQ-14–947 and SQ-14–948 are most closely related to Nocardia arthritidis while secA1 supported placement next to N. exalbida and N. gankensis. Both of these trees, however, had low bootstrap support (<50) at the basal nodes indicating unreliable branching patterns (Fig. 2A, 2).) For the MLST analysis, the bootstrap support values were >57 at all nodes, and SQ-14-947 and SQ-14-948 are monophyletic with Nocardia arthritidis (Fig. 2B). The 16S rRNA alignment contained only 49 informative positions (out of 1489 total positions) while the MLST consensus sequence alignment contained 123 informative positions (out of 2375 total positions) illustrating the increased resolution of the MLST approach. Pairwise distance comparisons of concatenated MLST sequences: 1) SQ-14-947 and SQ-14-948.

### Table 1

| Nocardia species included in the MLST phylogenetic analysis. |
|-------------------------------------------------------------|
| Species                                             | Strain Name | Accession Number |
| Nocardia arthritidis                                    | NBRC 100137 | BDB0100000841 |
| Nocardia beijingensis                                   | NBRC 16342 | BDB0100000221 |
| Nocardia brasiliensis                                   | ATCC 700358 | NC_0168611 |
| Nocardia brevicaena                                    | NBRC 12119 | NC_0186000241 |
| Nocardia cyriacigeorgica                               | GUH-2 | NC_0168871 |
| Nocardia exalbida                                       | NBRC 100660 | NC_BAF20100000281 |
| Nocardia farcinica                                      | IFM 10152 | NC_0063611 |
| Nocardia gankensis                                      | NBRC 108242 | BDBM0100000061 |
| Nocardia pneumonia                                     | NBRC 100136 | NC_BAF1010000211 |
| Nocardia tenerifensis                                  | NBRC 100105 | NC_BAF10000001461 |

* Gene sequences were extracted from publicly available genomes in the GenBank database at NCBI.
948 isolates were identical (100% identity); 2) with 12/2389 variable sites between SQ-14-947 and Nocardia arthritidis (99.5% identity); 3) with 22/2389 variable sites between SQ-14-947 and Nocardia beijingensis (99.1% identity); 4) with 25/2389 variable sites between SQ-14-947 and Nocardia exalbida (99% identity); and 5) with are 38/2389 variable sites between SQ-14-947 and Nocardia beijingensis (98.4% identity). These results showed via MLST that both isolates were identified as Nocardia arthritidis.

Discussion

Greater than 80 valid species of Nocardia have been described (List of Prokaryotic Names, www.bacterio.net, accessed 22 April 2014), with only 11 species reported as isolated from human infections to include N. abscessus, N. brasiliensis, N. cyriacigeorgica, N. farcina, N. nova, N. otitidiscaviarum, N. paucivorans, N. pseudobrasiliensis, N. transvalensis, N. veteran, and N. wallacei [13]. Initially, Nocardia species that cause human infection were typically classified as N. asteroides or N. asteroides complex. But with the advent of molecular analysis for more precise identification, N. asteroides complex became an obsolete species [14]. Overall, Nocardia species are typically associated with environmental sources [13]. The Nocardia species are long thin, finely beaded branching gram-positive bacilli that are strict aerobic, generally modified acid-fast positive and typically stain positive by the Gomori methanamine silver (GMS) stain in tissue [4]. Amplification and sequencing of individual conserved genes, such as the 16S rRNA gene, hsp65 gene, secA gene, and gyrB gene have been used for the identification of Nocardia species [4,7,14]. Genomic sequencing has led to the discovery of numerous previously unrecognized or rare Nocardia species that cause human disease. This report describes a case of disseminated infection by Nocardia arthritidis within the United States and highlights the methods used to verify the species identification.

Nocardia arthritidis was first published as a valid species in 2004 by Kageyama et al., who reported a Japanese patient from whom N. arthritidis type strain IFM 10035T (NBRC 100137T, JCM 12120T, DSM 44731T) was isolated from the sputum of a patient with rheumatoid arthritis [15]. This isolate was classified by the 16S rRNA sequence, DNA–DNA hybridization, and biochemical phenotypic properties [15]. Since this initial publication, additional cases caused by N. arthritidis have been reported to include a recovery of N. arthritidis from a frontal lobe lesion in a patient in Spain with a history of silicosis [16]; a case of cerebral nocardiosis in an immunosuppressed patient with systemic lupus erythematosus in Turkey [17]; and a case of N. beijingensis and N. arthritidis coinfection in a retrotracheal necrotic mass of an immunosuppressed patient in Ohio, USA [18]. Each of these reports primarily utilized 16S rRNA sequencing or species identification [16–18]. Conditions that contribute to the risk for nocardiosis include cellular immune dysfunction, usually due to high-dose steroids, hematologic malignancy (such as CLL in the patient presented here), or chronic immune suppression following solid organ transplantation [19–21] (Rosman article, 21, 22). Chronic obstructive pulmonary disease may also be a risk factor, and up to a quarter of nocardiosis cases are noted in immunocompetent individuals [19,20]. The present case patient was treated with prednisone (5 mg per day) for RA, a dose that is not considered to be immunosuppressive, although corticosteroid use has been shown to correlate with the development of pulmonary or disseminated nocardiosis in 64.5% (n = 20 of 31) of nocardiosis cases [19,20].

Regardless of immune status there is a risk for progression to disseminated disease in about 30% of all patients with primary nocardiosis [20]. Disseminated infection is characterized by abscess formation at multiple body sites with common sites being the brain, retina, skin, subcutaneous tissue, kidney, bone and heart [19]. The criteria for disseminated nocardiosis include involvement of two or more noncontiguous site organs [22]. Nocardia species reported associated with disseminated nocardiosis included N. abscessus, N. beijingensis, N. cyriacigeorgica, N. farcina, N. higoensis, N. nova, N. otitidiscaviarum, N. paucivorans, N. pseudobrasiliensis and N. transvalensis [1]. CNS involvement has been noted in 11.7–44% of systemic nocardiosis cases, usually in the form of one or more brain abscesses [6,19,23].

In the present case, N. arthritidis was isolated from tissue obtained from both the lung and leg in pure culture from a febrile patient with a history of CLL and RA. The original modified acid fast (FITE) stain of a fine-needle biopsy of the RLL was reported as
negative for bacteria. Following a positive culture, the original FITC-stained tissue slides were re-observed using 1000× magnification. Subsequently, rare filamentous modified AFB negative bacteria were discovered. Due to a lack of contrast between the organism and background, the organisms were not visible under 40× magnification (standard procedure used to observe histology slides). Over-decolorization during the staining process of the rare finely beaded Nocardia cells present in the sample may also have accounted for the inability to observe the filamentous bacteria in the original stain. A positive control slide (containing a Mycobacterium species) that was used at the time of staining the original specimen correctly stained as modified AFB positive. A modified Ziehl-Neelsen acid-fast stain performed from culture of the isolate was also positive. The GMS stained slide of the original biopsy sample remained negative for both bacteria and fungal cells after observation under 1000× magnification. Rare reports have shown that a negative GMS stain was seen for some Nocardia species although the reason for this was not elucidated [24,25].

Molecular sequencing of the variable regions of the 16S rRNA gene, hsp65 gene, secA gene, and gyrB gene targets have been shown to be discriminatory enough to distinguish among closely related Nocardia species [1,4–8]. Historically, the AST pattern type groups were utilized to separate among species and complexes within the Nocardia genus. The six major drug pattern groups have been classified as Types I–VI [1]. As indicated previously, N. abscessus belongs to the AST pattern designated as Type I; whereas, N. beijingensis and N. arthritidis do not have officially accepted AST patterns [1,26]. Although the AST patterns for Nocardia can remain useful in the initial characterization of Nocardia species, inconsistencies in this pattern recognition have been noted and are now used less frequently than molecular methods. In conclusion, this report describes a unique case of disseminated nocardiosis caused by N. arthritidis. The difficulty of recognizing Nocardia species in tissue and the complexity of methods needed to provide a confirmed species identification were shown. The benefit of utilizing MLST for difficult to speciate is described in this report. Increased use of molecular methods and the expansion of mass spectrometry for identification purposes will likely expand the number of Nocardia species associated with human disease. Consequently, human infections linked to rarely detected Nocardia species such as N. arthritidis and newly described species will likely be encountered more frequently from a wider geographical distribution. Finally, the observation of tissue following histological staining using high-power magnification is suggested when negative results are seen from patients who are at high risk for nocardiosis.

Conflict of interest

All authors report no conflicts of interest.

References

[1] Brown-Elliott BA, Brown JM, Conville PS, Wallace Jr. BJ. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. Clin Microbiol Rev 2006;9(2):259–82.
[2] Validation list no. 113. List of new names and new combinations previously effectively, but not validly, published, Int J Syst Evol Microbiol 57( Pt 1) (2007) 1.
[3] Le Roes M, Meyers PR. Nocardia gamkensii sp. nov. Antonie Van Leeuwenhoek 2006;90(3):291–8.
[4] Conville PS, Witebsky FG. The complexity of Nocardia taxonomy: implications for the clinical microbiology laboratory. Clin Microbiol News 2010;32(16):119–25.
[5] Conville PS, Zelazny AM, Witebsky FG. Analysis of secA1 gene sequences for identification of Nocardia species. J Clin Microbiol 2006;44(8):2760–6.
[6] Lerner PI. Nocardiosis. Clin Infect Dis 1996;22(6):891–903.
[7] Rodriguez-Nava V, Cuable A, Devalder G, Flandrois P, Boiron P, Laurent F. Use of PCR-restriction enzyme pattern analysis and sequencing database for hsp65 gene-based identification of Nocardia species. J Clin Microbiol 2006;44(2):336–46.
[8] Wauters G, Avesani V, Charlier J, Janssens M, Vaneechoutte M, Delmee M. Distribution of nocardia species in clinical samples and their routine rapid identification in the laboratory. J Clin Microbiol 2005;43(6):2624–8.
[9] A. bio-systems. Fast Microseq 500 16S rRNA Bacterial Identification Kits Protocol. Foster City, CA, USA: Life Technologies Corporation; 2010.
[10] Interpretive criteria for identification of bacteria and fungi by DNA target sequencing. Approved Guideline, CLSI. Wayne, PA, USA: CLSI Institute; 2008.
[11] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4(4):406–25.
[12] Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc 2012;87(4):403–7.
[13] Parte AC. LPSN–list of prokaryotic names with standing in nomenclature. Nucleic acids research 2014;42(Database issue):D613–6.
[14] Reddy AK, Garg P, Kaur I. Speciation and susceptibility of Nocardia isolated from ocular infections. Clin Microbiol Infect 2010;16(8):1168–71.
[15] Kaygama A, Torikoe K, Iwamoto M, Masuyama J, Shibuya O, Okazaki H, et al. Nocardia arthritidis sp. nov, a new pathogen isolated from patient with rheumatoid arthritis in Japan. J Clin Microbiol 2004;42(6):2366–71.
[16] Tamarit M, Poveda P, Baron M, Del Pozo JM. Four cases of nocardial brain abscess. Surg Neurol Int 2012;3:88.
[17] Yildiz O, Alp E, Tokgoz B, Tucer B, Aygen B, Sumerkan B, et al. Nocardiosis in a teaching hospital in the Central Anatolia region of Turkey: treatment and outcome. Clin Microbiol Infect 2005;11(6):495–9.
[18] Aragaki-Nakahodo A, Benzaquen S, Kirschmer M. Coinfection by Nocardia beijingensis and Nocardia arthritidis in an immunocompromised patient diagnosed by endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA). Respir Med Case Rep 2014;12:22–3.
[19] Sorrell TC, Mitchell DH, Iredell JR, Chen SCA. Nocardia Species, Mandell, Douglas, and Bennett’s Principles and Practice of Infectious Diseases. Philadelphia, PA: Churchill Livingstone Elsevier; 2010. p. 3199–207.
[20] Martinez Tomas R, Menendez Villanueva R, Reyes Calzada S, Santos Durantez M, Valles Tarazona JM, Modesto Alapont M, et al. Pulmonary nocardiosis: risk factors and outcomes. Respirrology 2007;12(3):394–400.
[21] Rosenthal T, Rosenmann E, Tomaasoni D, Amenta F. Effect of lercanidipine on kidney microanatomy in Cohen-Rosenthal diabetic hypertensive rats. J Cardiovasc Pharmacol Ther 2007;12(2):145–52.
[22] Simpson GL, Stimson EB, Egger MJ, Remington JS. Nocardial infections in the immunocompromised host: a detailed study in a defined population. Rev Infect Dis 1981;3(3):492–507.
[23] Beaman BL, Beaman L. Nocardia species: host-parasite relationships. Clin Microbiol Rev 1994;7(2):233–64.
[24] Luu CA, Vadmal MS. Case report Nocardia asteroides mycetoma. Ann Clin Lab Sci 2001;33(3):329–33.
[25] Karatowskaia S. Reatment of nocardia higoensii/shinofusenius masquerading as bronchitis and presenting as a solitary mass in a bone marrow transplant recipient. Chest J 2012 (4, MeetingAbstracts).
[26] CLSI. Susceptibility testing of Mycobacteria, Nocardiae, and other Aerobic Actinomycetes; Approved Standard-Second Edition, CLSI M24-A2, CLSI, Editor 2011, Wayne, PA, 2011.
[27] Coisy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 2010;27:2214–1.