



Necrotizing Pneumonia and Empyema in an Immunocompetent Patient Caused by *Nocardia cyriacigeorgica* and Identified by *16S rRNA* and *secA1* Sequencing

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Nocardiosis is a rare and potentially life-threatening infection. *Nocardia species* are a group of aerobic actinomycetes, which are filamentous, branching, gram-positive, and acid-fast bacilli [1]. Worldwide, respiratory and disseminated infections are most often due to *N. asteroides* complex, which is increasingly recognized as an opportunistic infection in immunocompromised hosts with underlying HIV infection, neoplastic disease, or long-term high-dose corticosteroid therapy [2]. Recently, infection of *N. cyriacigeorgica* was reported in an immunocompromised patient in Korea, which was identified by *16S rRNA* sequencing analysis and additional biochemical tests combined to draw a conclusion [3]. Here, we describe a case of necrotizing pneumonia and empyema caused by *N. cyriacigeorgica* in a 77-year-old Korean male patient who was immunocompetent. This case was diagnosed by DNA amplification and sequence analyses of the *16S rRNA* and *secA1* genes.

The patient was referred to our hospital from a local clinic because of dyspnea and right-side chest pain after he had a fall from a bed 2 days before presentation. He had no underlying medical problems except controlled hypertension. On initial clinical

examination in the emergency room, oxygen saturation was 89.7%, but it improved to 97% after initiation of 5 L oxygen. The patient had normal vital sign and electrocardiogram. Chest radiography revealed a pleural effusion on the right side (Fig. 1A). The pleural effusion was drained by chest tube placement, and it was reddish and had many red blood cells (RBCs) (>10,000/L) and 850 leukocytes/L (96% of polymorphonuclear cells). Laboratory investigation showed a leukocyte count of 6,900/L with 97.2% segmented neutrophils and elevation of serum C-reactive protein level of 27.8 mg/dL, erythrocyte sedimentation rate of 53 mm/hr, and serum lactate dehydrogenase of 575 IU/L. *Mycobacterium tuberculosis* PCR performed using the pleural fluid isolate tested negative. Chest computed tomography (CT) scan with contrast showed pleural effusion of both lungs with pleural thickening (Fig. 1B). Pleural fluid cytology was negative for malignant or severe inflammatory changes. Whole body fusion positron emission tomography (PET)/CT showed diffuse hypermetabolism of both pleural surfaces, but it was more suggestive of an inflammatory lesion than malignancy. The patient was clinically diagnosed as having both necrotizing pneumonia and he-

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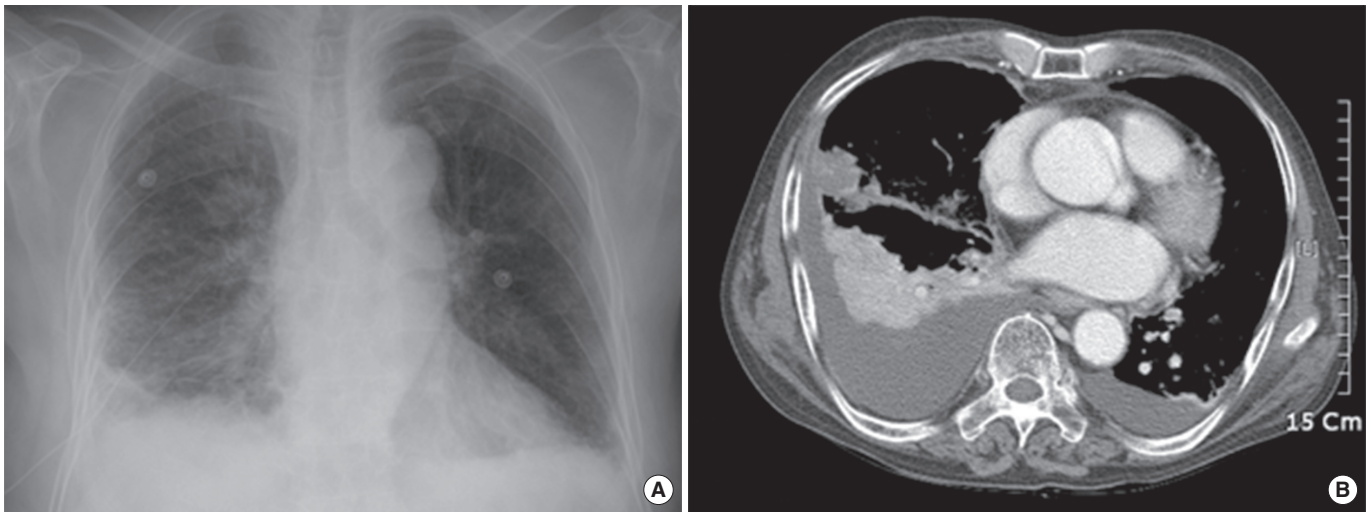


Fig. 1. (A) Both chest radiograph (A) and computed tomography scan of the thorax (B) demonstrate bilateral pleural effusions.

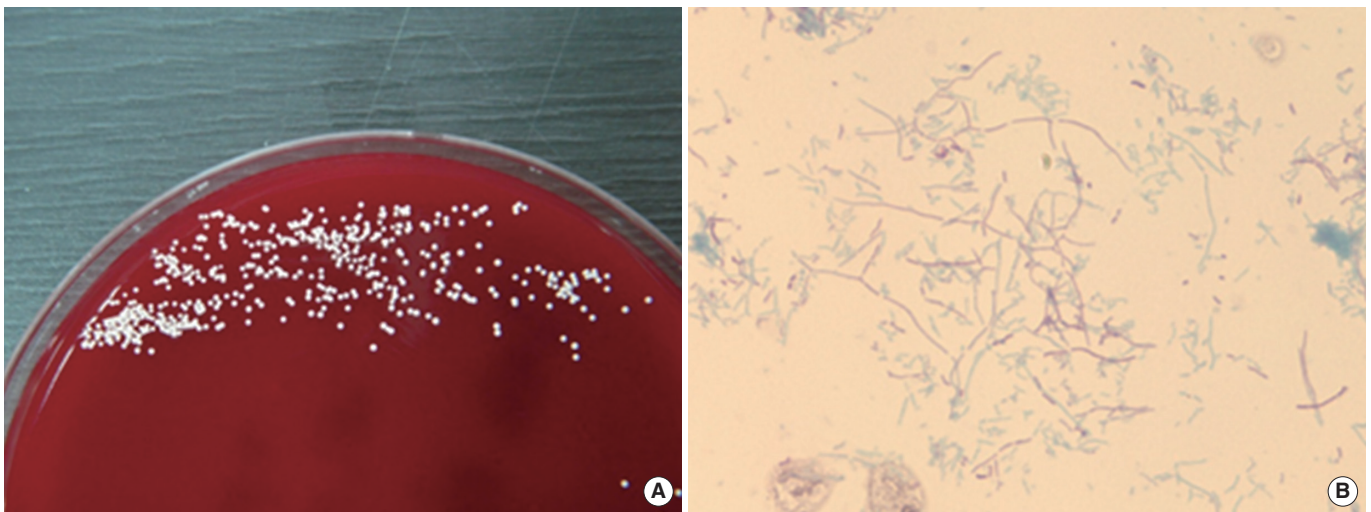


Fig. 2. Colony morphology on sub-inoculation and microscopic morphology. (A) Chalky-white colonies on blood agar plates at 36°C in 5% CO₂. (B) Beaded, branching, gram-positive rods of *N. cyriacigeorgica* (modified acid-fast bacilli stain, ×400).

mothorax and was empirically treated intravenously with a third-generation cephalosporin and aminoglycoside. Although fever was observed on admission day 7 and aggravation of pneumonia was noted on a follow-up CT scan taken on day 15, no organism was obtained from subsequent sputum, pleural fluid, or blood cultures. On day 17, the patient was transferred to the intensive care unit and received ventilator support because of altered mental status and respiratory distress. On day 23, pleural fluid cultures on sheep blood agar plates (BAP) grew rough, chalky-white colonies with an earthy odor; the presence of abundant thin, modified acid-fast, and gram-positive branching filaments was observed on microscopic examination (Fig. 2). The same organism was isolated once again from pleural fluid; thus, the

patient was suggested to have a nocardial infection. We decided to obtain further information using molecular methods for *16S rRNA* and *secA1* sequence analysis. However, multidrug-resistant *Acinetobacter baumannii* was identified from the patient's sputum obtained on day 36, and the patient died from progression of hospital/ventilator-associated pneumonia and respiratory failure. The etiologic diagnosis was established post-mortem by means of molecular analysis.

To identify the bacterium, molecular identification was performed by DNA amplification and sequencing analysis of the *16S rRNA* and *secA1* genes from the chest tube drainage isolate. *16S rRNA* and *secA1* gene fragments were amplified by standard methods according to CLSI guidelines [4]. The *secA1*

protein is an essential component of the preprotein translocase ATPase, which provides the driving force for the export of proteins across the bacterial cytoplasmic membrane [5]. The *secA1* locus has potential for nocardia species identification as an adjunct to 16S rRNA gene sequencing [6]. Two sub-regions of the 16S rRNA and *secA1* genes were amplified and sequencing analysis using the previously reported primer pairs [4, 6]. Purified PCR products were sequenced directly using the BigDye Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems; Foster City, CA, USA) on an ABI Prism 3100 genetic analyzer (Applied Biosystems). A GenBank Basic Local Alignment Search Tool (BLAST) search revealed that the 16S rRNA gene sequence of the isolate showed 100% and 98% homology of 695 base pairs to corresponding sequences of the previously sequenced *N. cyriacigeorgica* and *N. farcinica* (GenBank accession numbers NR_074699.1 and AB162791.1). Moreover, for the *secA1* gene, we confirmed the corresponding sequence from *N. cyriacigeorgica* (JQ773455.1) with 100% homology of 505 base pairs, whereas less than 94% homology was noted for other *Nocardia* species. For further phylogenetic analysis, 16S rRNA and *secA1* gene sequences isolated from the chest tube drainage fluid were aligned using ClustalW [7]. Phylogenetic trees were constructed with the neighbor-joining method using the MEGA 5 software package (<http://www.megasoftware.net>) and Kimura 2 parameters as the substitution model [8]. Statistical significance of the phylogenies was assessed by bootstrap analysis with 1,000 pseudoreplicate datasets. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Fig. 3).

Prior to the advent of molecular biological techniques, traditional diagnostic methods for identifying these organisms were often ambiguous. Rapid and accurate identification of aerobic actinomycetes is becoming increasingly important in clinical microbiology laboratories. Pulmonary nocardiosis may clinically be acute, subacute, or chronic. Acute nocardiosis often occurs in severely immunocompromised patients and correlates with a poor prognosis, with a high mortality of more than 40% in cases of disseminated disease. Subacute lung nocardiosis, the most common clinically encountered form, often mimics tuberculosis, pneumocystosis, invasive fungal infection, and/or malignancy [9]. Currently, the *Nocardia* genus has approximately 100 reported species of different taxonomic statuses (<http://www.bacterio.cict.fr/n/nocardia.html>). According to Wallace et al. [10], the heterogeneity of the *Nocardia* group in terms of drug susceptibility patterns requires frequent new taxon formation to account for new species. Of the known *Nocardia* species, the species most

frequently involved in human infection are members of the *N. asteroides* complex. The members of this complex have been sub-classified into 6 separate groups according to drug susceptibility patterns with numerous new species described [11]. *N. cyriacigeorgica* was first characterized in 2001 by Yassin et al. [12] from the bronchial secretions of a patient with chronic bronchitis. At that time, this organism was identified as a novel species that was both molecularly and biochemically different from previously described members of the genus *Nocardia*. It corresponds to strains of the drug pattern type VI of the *N. asteroides* complex. They reported that this new species had different DNA-DNA hybridization with *N. abscessus* (45% reassociation) and *N. asteroides* complex type strains (61% reassociation).

Nocardia species do not typically affect immunocompetent hosts. Clinically oriented criteria for nocardiosis may be useful for evaluating the significance of a positive culture from isolated specimens. Criteria include direct visualization of a rod-form microorganism with gram-positive and acid-fast microscopic findings and repeated isolation from serial clinical samples in immunocompromised hosts. However, their presence should suggest a careful evaluation of clinical conditions. In a retrospective study, 20% of *Nocardia* species isolates were found not to be associated with the clinical disease [13]. Most nocardiosis cases are not rapidly recognized because of lack of pathognomonic clinical signs, limitations of conventional biochemical tests for slow-growing bacteria, and the presence of commensal flora on culture. In the present case, when gram-positive bacilli were isolated for the first time from the pleural fluid of our patient, we considered that this strain was present owing to the transient colonization in response to chest tube replacement. However, subsequent pleural fluid cultures obtained the same results, along with aggravation of the patient's clinical status and the lack of any other documented organism. The strain was presumed to be a member of the genus *Nocardia*, and further molecular analysis was planned. For therapy, *Nocardia*, and especially *N. cyriacigeorgica*, is typically susceptible to sulfonamide, amikacin, linezolid, imipenem, and broad-spectrum cephalosporins, which are often used empirically in the absence of antibiotic susceptibility information [14]. In this study, the strain was tested for susceptibility using the Sensititre RAPMYCOI plate (Trek Diagnostic Systems, Cleveland, OH, USA) according to CLSI guidelines [15]. The isolate was susceptible to trimethoprim/sulfamethoxazole (minimum inhibitory concentration [MIC]: 0.5/9.5 µg/mL), amikacin (≤ 1 µg/mL), linezolid (≤ 1 µg/mL), ceftriaxone (≤ 4 µg/mL), minocycline (≤ 1 µg/mL), and tobramycin (≤ 1 µg/mL), and it was resistant to ciprofloxacin (>4 µg/mL), moxifloxa-

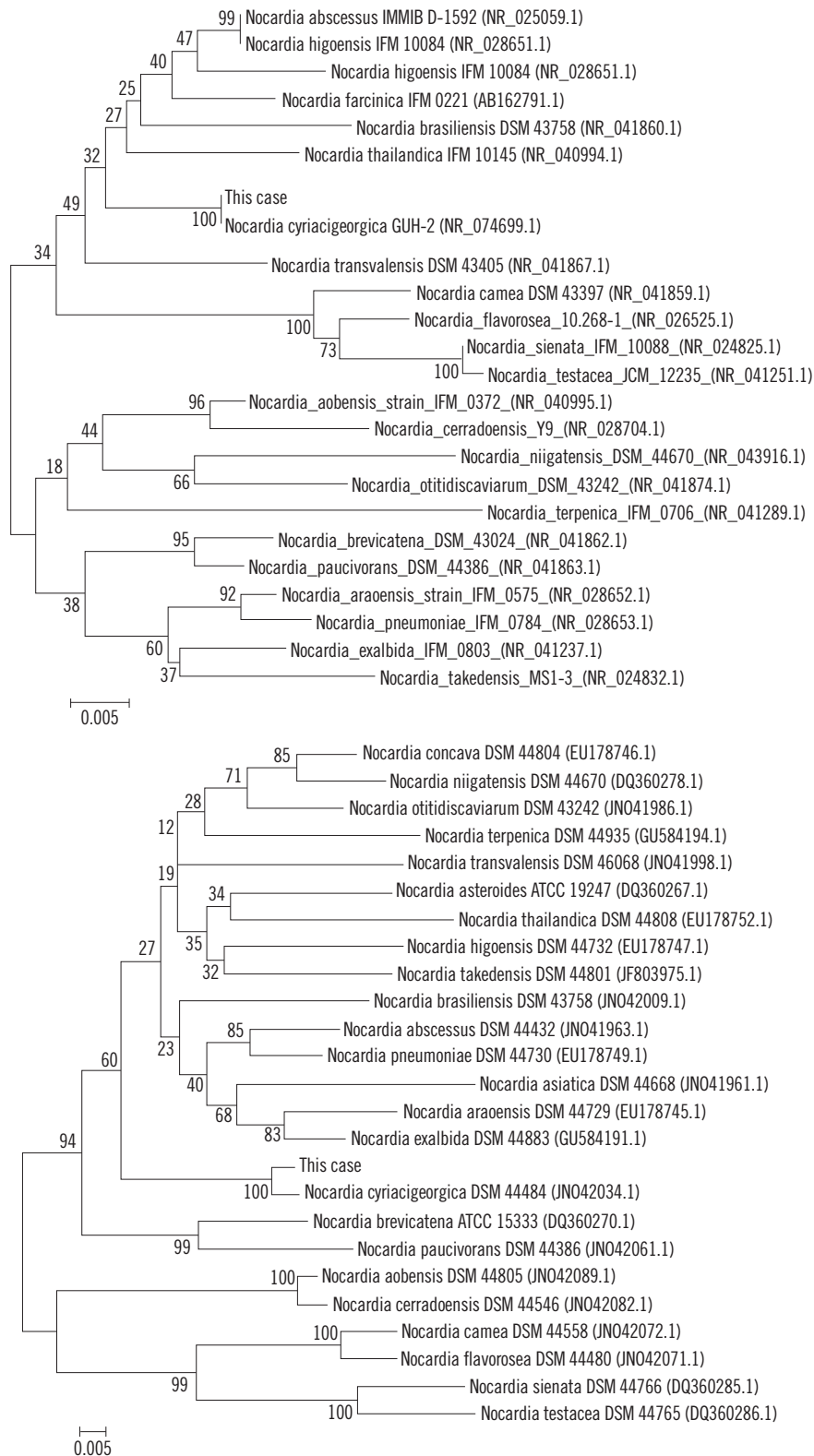


Fig. 3. Molecular phylogenetic tree constructed by the neighbor-joining method by using (A) *16s rRNA* and (B) *secA1* gene sequences from the *N. cyriacigeorgica* isolate and various *Nocardia* species. Reference sequences indicating the species strain and accession numbers are given in parentheses. All names and accession numbers are as cited in GenBank. Scale bar represents 5 nucleotide substitutions per 1,000 nucleotides.

cin (4 µg/mL), imipenem (64 µg/mL), cefepime (32 µg/mL), and amoxicillin/clavulanic acid (64/32 µg/mL). Although the patient received empirical treatment with third-generation cephalosporin and aminoglycoside, he died from progression of hospital/ventilator-associated pneumonia and respiratory failure.

Application of molecular methods for sequence analysis of the *16S rRNA* and *secA1* genes can assist in a quicker diagnosis of most *Nocardia* species. For certain groups of aerobic actinomycetes, the use of alternative gene targets such as the *secA1* gene can serve as adjuncts to *16S rRNA* sequencing of *Nocardia* species. The use of targeted genes is useful because some species may share 99.6-100% *16S rRNA* sequence homology, despite being different species [16, 17]. Thus, definitive identification was obtained by *16S rRNA* analysis and alternate DNA target analysis with the *secA1* gene.

Although this is the second case of pulmonary nocardiosis caused by *N. cyriacigeorgica* in Korea [3], to the best of our knowledge, this is the first report on an immunocompetent patient with *N. cyriacigeorgica* infection, which was identified using molecular analysis for *16S rRNA* and *secA1* sequences. This case shows that necrotizing pneumonia and empyema can occur in an immunocompetent patient and should be considered in the differential diagnosis of suspected cases.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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