

A Case of Pulmonary Nocardiosis Due to *Nocardia cyriacigeorgica*

Nocardia cyriacigeorgica'nın Etken Olduğu Bir Akciğer Enfeksiyonu Olgusu

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Geliş Tarihi/Received: 08.01.2014
Kabul Tarihi/Accepted: 08.05.2014

This study was presented as a poster at 1st
National Clinical Microbiology Meeting,
12-16 November 2011, Antalya

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ABSTRACT Nocardiosis is an acute or chronic infectious disease caused by filamentous bacteria belonging to the genus *Nocardia*. *Nocardia* species opportunistically infect both immunocompromised and immunocompetent individuals. The lungs are the primary site of infection. It is often difficult to treat and the mortality is high. *Nocardia cyriacigeorgica* has been described as an emerging pathogen. We present a case of 83-year-old female intensive care unit patient, with prediagnosis of pneumonia, hypertension and chronic obstructive pulmonary diseases. Pulmonary infection was caused by *Nocardia cyriacigeorgica*. The identification included conventional and molecular sequencing tests. According to DNA sequence analysis 16S rRNA was >99% compatible with *Nocardia cyriacigeorgica*. Infection was successfully treated with trimethoprim-sulfamethoxazole.

Key Words: Nocardia infections; sequence analysis, DNA

ÖZET Nokardiyoz, *Nocardia* cinsi filamentöz bakterilerin etken olduğu enfeksiyonlardır. Enfeksiyonlar akut veya kronik seyredebilir. *Nocardia* türleri özellikle immünkompromize konakta fırsatçı enfeksiyonlara neden olurken, immünkompetan konakta da enfeksiyon etkeni olarak tanımlanabilirler. Enfeksiyon primer olarak akciğerleri tutar. Bazen tedavide zorluklar yaşanır ve yüksek mortalite oranlarına sebep olur. *Nocardia cyriacigeorgica*, nadir görülen bir patojendir. Bu olgu raporunda pnömoni, hipertansiyon, kronik obstrüktif akciğer hastalığı ön tanıları ile yoğun bakım servisinde yatmakta olan 83 yaşında kadın hasta sunulmuştur. Pnömoni etkeni olarak *Nocardia cyriacigeorgica* tespit edilmiştir. İdentifikasyonu konvansiyonel ve moleküler sekans testleri ile yapılmıştır. DNA dizi analizi sonucunda, bakteriye ait 16S rRNA gen dizisinin >%99 oranında *Nocardia cyriacigeorgica* ile uyumlu olduğu tespit edilmiştir. Trimetoprim-sulfametoksazol tedavisi ile hasta iyileşmiştir.

Anahtar Kelimeler: Nokardiya enfeksiyonları; dizi analizi, DNA

Türkiye Klinikleri J Case Rep 2015;23(3):275-8

N*ocardia* species belong to aerobic family Actinomycetaceae. Aerobic actinomycetes are divided into two sections such as *Nocardia* and other than *Nocardia* bacteria. *Nocardia* spp. are common in nature in earth and waters and they are mostly saprophytic bacteria. There are 70 known *Nocardia* species. More than 25 of them are describes as human isolates and many others are pathogenic in animals.¹

Nocardiosis, are a life-threatening diseases that could progress locally or systemically as a opportunistic infection which are caused by bacteria belonging to *Nocardia* species. Especially patients with malignity, chronic

doi: 10.5336/caserep.2014-38732

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pulmonary diseases, AIDS (acquired immune deficiency syndrome), solid organ transplant recipients, patients treated with corticosteroid and immunosuppressive drugs and patients with history of surgery, intervenes catheter intervention and trauma are in risk group. Systemic Nocardiosis are very common diseases worldwide. Lung, skin, soft tissue, central nervous system (CNS) are the most common clinic forms of this diseases. Especially pulmonary Nocardiosis which is developed by inhalation is the most common form. *N. asteroides* is the most common isolated species. In systemic infection by *N. asteroides* lesions which look like false *Mycobacterium tuberculosis* are formed in lung and pleurae.^{2,3}

Nocardia spp. is a facultative intracellular pathogen which can grow macrophages and polymorphonuclear leukocytes (PMNL). Generally *Nocardia* species are microorganisms with branched filaments which can sometimes represent forms like bacillus or cocci. They are Gram positive bacteria. While some species can stain acid resistant in some growth stages, others don't have this feature.

Nocardia species are classified according to clinic features. Wallace et al. described variations in the drug susceptibility patterns of 78 isolates determined biochemically to be *N. asteroides*.⁴ Based largely on molecular testing microorganisms which phenotypically represent *N. asteroides* are divide into six different drug pattern types and one extra group. Various species such as *N. farcinica* (drug pattern type V), *N. nova* (drug pattern type III), *N. abscessus* (drug pattern type I) and drug pattern types II, IV, and VI are defined in this set. *N. abscessus* (drug pattern type I) is susceptible to ampicillin, amoxicillin-clavulanate, ceftriaxone, linezolid, amikacin mostly resistant to imipenem and definitely resistant to ciprofloxacin and clarithromycin. *N. asteroides complex* (drug pattern type VI) is resistant to ampicillin, amoxicillin-clavulanate, clarithromycin, erythromycin and ciprofloxacin where as it is susceptible to amikacin, cefamandole, ceftriaxone, cefotaxime, linezolid and imipenem. Before its proper identification *Nocardia cyriacigeorgica* belong to drug pattern type VI *Nocardia* genus.^{5,6} *Nocardia cyr-*

iacigeorgica was first characterized in 2001 by Yassin et al. as a novel species differing by molecular and biochemical characteristics from the previously described members of the genus *Nocardia*.⁷ Kageyema et al. using sequence analysis of 121 isolates identified as *N. asteroides* from Japan and Thailand showed 27 of these isolates (22.3%) to have 16S r RNA gene sequences identical to that of the type strain of *N. cyriacigeorgica*.⁸ In addition, isolates of *N. cyriacigeorgica* have been identified by 16S r RNA gene sequence analysis from patients with disseminated disease in Canada, France, Greece and The Netherlands.⁹⁻¹³ In these paper we described a pneumonia case in which *N. cyriacigeorgica* is causative agent.

CASE REPORT

83 years old female patient complaining severe cough and respiratory failure was admitted to our hospital. Patient was accepted to intensive care unit with prediagnosis of pneumonia, hypertension and chronic obstructive pulmonary diseases. In chest X ray appearance compatible with infiltration was seen. In thorax computed tomography (CT) pleural effusion, ground glass appearance in left and right lung and nodules with calcification in lower lobe of left lung were determined. Patient had fever of 38°C and leukocytosis (16 400/mm³, 89% neutrophil) and oxygen saturation 80% patient was diagnosed with bacterial pneumonia. Empiric ampicillin and moxifloxacin therapy was started. Sputum specimen send to microbiology laboratory for tuberculosis culture. Sputum specimen was homogenized and decontaminated before inoculating Lowenstein-Jensen (LJ) medium. Culture was incubated at 37°C. Growth was detected in seventh day by daily routine examinations. Specimen was stained with Erlich-Ziehl Neelsen (EZN). Partially acid-fast beaded branching filaments were seen. Colonies grown in LJ were subcultured to human blood agar and incubated at 37°C. After 24 hours very faint, thin growth was detected. After 48 hours colonies dry, opaque white with powdery appearance having distinctive odor of moist basement were seen. In Gram stain Gram positive hyphae like bacilli were determined. In modify EZN

stain acid fast thin branching bacilli were observed. In order to determine if the isolate was *Mycobacterium spp.* polymerase chain reaction (PCR) test with universal mycobacterium primer were done. By this molecular test we detected that it was not *Mycobacterium spp.* Then DNA sequence analysis was performed. After DNA extraction 800bp gene was amplified by using universal primers (fD1 and 800R) targeting 16S rRNA gene. DNA sequence analyses were done double sited at CEQ2000XL automated DNA sequence analyzers (Beckman Coulter) by using fD1 and 610R primers. The 500 bp DNA sequence determined in the study was compared with gene bank data base by using online BLAST program (<http://blast.ncbi.nlm.nih.gov/>). According to analysis 16S rRNA was >99% compatible with *N. cyriaciageorgica*. After this laboratory diagnosis patient's antibiotics were discontinued and trimethoprim/sulfamethoxazole (SXT) treatment was started. In follow up patient did not have fever, leukocytosis, oxygen saturation was raised to 90%. In control direct lung X ray significant improvement in infiltrations were observed.

DISCUSSION

Recently *Nocardia* infections are occurring with increased frequency. *Nocardia* infection is known as an opportunistic infection in immunocompromised hosts but it consists at least 15% of the infections in patients without a definable predisposing condition.¹⁴ It is obvious that in patients with underlying diseases like chronic granulomatous disease (CGD), chronic obstructive pulmonary diseases (COPD), corticosteroid, diabetes mellitus (DM), pulmonary alveolar proteinosis, Systemic lupus erythematosus, *Nocardia* remains as a cause of complicated pulmonary and disseminated infection.¹⁵ In an observational study of all the patients diagnosed with pulmonary Nocardiosis over a 13-year period the predisposing conditions were defined as;

COPD (23%) transplantation(29%), HIV infection (19%), alcoholism (6.5%) and treatment with steroids (64.5%).¹⁶ Early identification and therapy of Nocardiosis is important for mortality especially among immunocompromised patients. In our case patient has a COPD as an underlying disease for pulmonary Nocardiosis.

Nocardia grow slowly on non-selective culture media. Isolation of macroscopically white R type colonies with powdery appearance which has typical odor of moist basement and microscopically Gram positive modify acid-fast bacilli with suspected clinic should be considered as *Nocardia* infection. For *Nocardia* species, classical phenotypically and biochemical methods may not be enough during identification at species level. *Nocardia* should be identified to species level because they have various different drug patterns. Also identification to species level is needed for epidemiological studies. For this reason molecular tests such as PCR, RFLP (Restriction Fragment Length Polymorphism) molecular analysis method, DNA sequence analyses, ribotyping are used. Gene sequencing of *Nocardia* species with this methods is important for taxonomy and detection of phylogenetically relationships within similar genus.¹⁷ *N. cyriaciageorgica* was previously four times isolated from patients pulmonary Nocardiosis in our country. In two of these cases, therapy with ceftriaxone, and amikacin combination was ineffective and patients were lost. One case was cured by ceftriaxone and amikacin combination and one case was cured by SXT without any complication.¹⁸ The case we are presenting is the fifth pulmonary Nocardiosis caused by *N. cyriaciageorgica* in our country and cured with SXT without any sequels. *N. cyriaciageorgica* is not a new organism, it was isolated from patients before but identified as *Nocardia asteroides*. By molecular studies, in recent years this organism is renamed.

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