

## Case Report

# Clinical utility of universal 16S rRNA PCR using sterile body fluids: lung abscess caused by *Nocardia farcinica*

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## Abstract

Nocardiosis is an infectious disease caused by Gram-positive rod-shaped bacteria and presents as a suppurative granulomatous disease in patients with compromised immune systems. Few studies have investigated the clinical utility of the universal 16S rRNA polymerase chain reaction (PCR) method using sterile body fluids for diagnosing nocardiosis.

A 64-year-old female patient was admitted to Chosun University Hospital with the complaint of fever. Computed tomography scans of her chest revealed the presence of empyema and an abscess in the right lung. Pus samples were collected using closed chest thoracostomy and were cultured. The results revealed the presence of Gram-positive bacilli, but the culture tests were unable to identify the causative microorganism. Despite antibiotic treatment, the patient died of the suspected empyema and abscess. Universal 16S PCR of her sterile body fluids in combination with sequencing was performed, which led to the diagnosis of *Nocardia farcinica* infection. Postmortem, the remainder of the pus samples cultured for 8 days confirmed the presence of *N. farcinica*. This study illustrates the importance of using routine universal 16S rRNA PCR with sterile body fluids to help diagnose atypical bacterial infections such as nocardiosis.

**Key words:** 16S rRNA; Gram-positive bacteria; polymerase chain reaction; Gram-staining; nocardiosis.

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## Introduction

Nocardiosis is an acute, subacute, or chronic suppurative granulomatous inflammation (SGI) caused by Gram-positive actinomycetes of the genus *Nocardia* [1].

Gram and acid-fast staining tests of clinical samples containing *Nocardia* reveal branching filaments of Gram-positive and acid-fast bacteria [2].

The majority of *Nocardia* infections are caused by the inhalation of bacteria. Lesions first appear in the lungs (pulmonary nocardiosis), and trauma to the skin can cause skin infections (skin/subcutaneous nocardiosis) [3]. Disseminated nocardiosis is caused by the hematogenous distribution of bacteria from the lungs (lesion), causing abscesses in the skin, brain, kidneys, spleen, and other organs/systems [3]. *Nocardia* can be cultured in 2–14 days [4].

As observed in the case of *Nocardia*, some bacteria are difficult to culture, hence take longer. The universal 16S rRNA polymerase chain reaction (universal 16S PCR) is a rapid and accurate diagnostic method for identifying such bacteria in sterile body fluids [5].

Chosun University Hospital routinely performs universal 16S PCR using sterile body fluids. Herein, we describe a case of *Nocardia farcinica* infection diagnosis using universal 16S PCR combined with sequencing.

## Case study

A 64-year-old female patient was admitted to the emergency room (ER) of Chosun University Hospital with a complaint of a fever. Three months before, she was admitted to the Department of Neurosurgery at the hospital and was treated for a subarachnoid hemorrhage (SAH). Following the SAH treatment, the patient remained bedridden at a convalescent care facility. On the day before her admission to the ER, she experienced a fever (38 °C), which led her to the hospital.

Apart from medication for diabetes and hypertension, the patient was not receiving any other medicine, such as immunosuppressive drugs. Respiratory examination during inhalation revealed coarse crackles on the right side of the chest.

Her vitals were as follows: blood pressure, 110/80 mmHg; heart rate, 120 beats/min; respiration rate, 18 breaths/min; body temperature, 37.8 °C. Computed tomography (CT) scans of the chest showed the presence of empyema and an abscess in the right lung (Figure 1).

A complete blood count (CBC) showed a leukocyte count of 5,190/mm<sup>3</sup>, hemoglobin of 7.5 g/dL, and a thrombocyte count of 174,000/mm<sup>3</sup>. HCO<sub>3</sub> concentration had increased to 30.9 mmol/L and pCO<sub>2</sub> and pO<sub>2</sub> concentrations were 39.1 and 71.9 mmHg, respectively. Moreover, pH and lactate levels had increased to 7.502 and 21.14 mg/dL, respectively.

Upon her admission to the hospital, treatment was initiated by administering piperacillin/tazobactam. Blood culture tests conducted at the time of admission showed no bacterial growth. On the second day after admission, pus samples were collected using closed tube thoracostomy and immediately cultured to identify the bacteria that caused the lung abscess and empyema. One week after admission, Gram's staining was performed, which revealed the presence of Gram-positive bacilli (GPB). The final culture tests were not successful, and no acid-fast bacilli cultures were obtained.

Universal 16S conventional PCR (C-PCR) was performed on the DNA samples extracted from the patient's lung abscess and empyema pus cells using

**Figure 1.** Chest CT scans of a 64-year-old female patient complaining of a fever at the time of her hospital visit and later diagnosed with nocardiosis showed a suspected abscess or empyema in her right lung.



DNeasy Blood & Tissue Kit (Qiagen, Seoul, Korea). Universal primers (27F, 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R, 5'-TACGGHTACCTTGTTACGACTT-3') targeting the 16S rRNA were used [6]. AmpliTaq Gold™ 360 Master Mix (Applied Biosystems) was used for PCR. Electrophoresis results confirmed the presence of positive bands. Sequencing was conducted at Cosmo Genetech (Daejeon, South Korea). BLASTN (National Center for Biotechnology Information) results showed 100% homology (1,307 bp) with *N. farcinica* type strain W6977 (accession no. CP031418).

Despite treatment, the patient continued to suffer from pancytopenia and fever and died 10 days after hospitalization. Universal 16S C-PCR was performed postmortem, which confirmed the presence of *N. farcinica*. The remainder of her pus samples obtained via closed tube thoracostomy were used for culture tests, which were conducted in blood culture bottles for more than 5 days. Bacterial cultures were detected on the 8<sup>th</sup> day. The successfully cultured bacteria were identified as *N. farcinica* with the help of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS, ASTA, Korea).

## Discussion

*Nocardia* (order: Actinomycetales; family: Nocardiaceae) are Gram-positive, rod-shaped [7], and partially acid-fast bacteria [2]. Majority of patients with *Nocardia* infections have an underlying disease affecting their immune system. In patients with no underlying disorders, trauma accounts for a majority of infections [2].

*Nocardia* infections are nonspecific, and the bacterial growth rate is slower than that of other bacteria. Bacterial colony identification in culture can, therefore, take as long as 2 weeks. Hence, dismissing cases of *Nocardia* infections is easy unless otherwise justified for suspecting its presence [8]. Preliminary analysis by the medical team ascribed the presence of Gram-positive rod-shaped bacteria in pus culture tests to previously administered antibiotics, which killed the causative bacteria. Thereafter, piperacillin/tazobactam was administered only in the suspected empyema and lung abscess cases caused by mixed microorganisms including Gram-positive bacteria. However, we confirmed the diagnosis of *N. farcinica* infection through universal 16S PCR testing after the patient's death.

*Nocardia farcinica* is associated with a high mortality rate even without immune deficiency. Hence, rapid identification and therapeutic intervention are of

utmost importance [9]. Since nocardiosis has no characteristic clinical symptoms [10], delayed diagnosis is common [11]. Therefore, PCR-based methods using sterile body fluids enable the speedy and accurate diagnosis of common bacterial infections even after the administration of antibiotics [9]. In a recent study involving 18 randomly selected hospitals and clinics in the Republic of Korea, a complete enumeration of automated blood culture systems was performed. In majority of the facilities, blood samples were automatically discarded when cultures could not be successfully obtained after 5 days [12]. A similar automated system of disposal is used in our hospital. In the present case report, approximately 8 days of culture were necessary to identify the bacterium as *N. farcinica* using the remainder of the pus samples. Therefore, it is highly unlikely that any hospital or clinic using these automated blood culture systems would reach the correct diagnosis of nocardiosis within 5 days of attempting initial culture growth.

If Gram-positive rod-shaped bacteria are detected from sterile samples (such as lung abscess and empyema), but the bacteria cannot be identified from culture tests, nocardiosis should be considered a possibility. A sufficient length of time should accordingly be allocated to the culture tests, and an effective testing method using sterile body fluids should be employed. We share our findings with clinicians and emphasize that the routinely used universal 16S PCR can be effective for diagnosing atypical bacterial infections such as nocardiosis.

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Written consent was obtained, and the study was approved and conformed to the correct standards.

### Ethics approval and consent to participate

The study was approved by the Ethics in Human Research Committee of Chosun University Hospital (IRB No. 2013-10-001-018). The patient provided written informed consent to participate in the study.

### Authors' contributions

All authors contributed to the study's conception and design. Manuscript draft and patient data analysis: Park JS and Seo JW. Drafting and Revising: Kim CM, Pyun SH, and Byung JY. Data Interpretation and Supervision: Yun NR. Conception and Design: Kim DM. All authors commented on

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